# Effects of alteration in liver blood flow and in hepatic enzyme induction on the pharmacokinetics of lignocaine and tocainide.

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# Effects of alteration in liver blood flow and in hepatic enzyme induction on the pharmacokinetics of Lignocaine and Tocainide

submitted by

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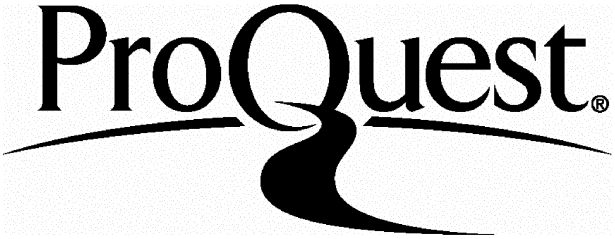
S. Supradist



TO MY PARENTS

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ABBREVIATIONS

| | |
|---|---|
| AUC | total area under the blood drug concentration-time curve |
| C | drug concentration |
| $C_o$ | concentration of drug in the blood at zero time |
| $C_t$ | concentration of drug in the blood at time t. |
| $C_s$ | concentration of drug in the blood entering the liver (systemic) |
| $C_{hv}$ | concentration of drug in the emergent venous blood (hepatic vein ) |
| $C_{\overline{ss}}$ | drug concentration in the blood at steady state |
| $C_{in}$ | concentration of drug in the blood entering the liver |
| $C_{out}$ | concentration of drug in the blood leaving the liver |
| CL | drug clearance |
| $CL_h$ | hepatic drug clearance |
| $CL_t$ | total body drug clearance |
| $CL_{int}$ | intrinsic hepatic drug clearance |
| $CL_{int(free)}$ | intrinsic hepatic free drug clearance |
| $CL_{int(tot)}$ | total intrinsic hepatic drug clearance |
| D | dose |
| E | hepatic extraction ratio |
| F | systemic availability of drug given orally |
| $f_B$ | ratio of the unbound drug concentration in plasma water to the whole drug blood concentration |
| $K_m$ | Michaelis-Menten constant |
| $k_e$ | first order rate constant for elimination of drug by all processes from central compartment |
| $k_{12}$ | first order rate constant for transfer of drug from central compartment to peripheral compartment |
| $k_{21}$ | first order rate constant for transfer of drug from peripheral compartment to central compartment |

| | |
|---|---|
| $k_{ab}$ | first order rate constant for absorption |
| $k_d$ | overall first order elimination rate constant for drug disposition |
| n | number |
| Q | hepatic blood flow |
| t | time |
| T | dosing interval |
| Vd | apparent volume of distribution |
| Vp | volume of central compartment |
| $Vd_{extrap}$ | apparent volume of distribution by extrapolation |
| $Vd_{area}$ | apparent volume of distribution by area |
| $Vd_{ss}$ | apparent volume of distribution at steady state |
| $V_{max}$ | maximum velocity of enzyme action |

subscripts

| | |
|---|---|
| i.v., p.o. | intravenous and oral routes |

subscripts

| | |
|---|---|
| 0, t | at time = 0 and t |

SUMMARY

The investigation was undertaken to observe the effects of alteration of liver blood flow and of liver metabolism on the kinetics of two antiarrhythmic drugs, lignocaine, a highly hepatic cleared drug and tocainide, a drug with low hepatic clearance in intact rats.

An initial study was undertaken to investigate the basic kinetics of both drugs following oral and intravenous administration at different dosage levels. Lignocaine was found to be the subject of "first pass" effect with a systemic availability of 0.019. Tocainide was found to be approximately 80% eliminated by the liver and approximately 20% by the kidneys. Systemic availability of tocainide was 0.94. The $t_{\frac{1}{2}}$ of lignocaine was found to be the same after intravenous and oral administration. The $t_{\frac{1}{2}}$ of tocainide was found to be longer after oral than intravnous administration.

From this preliminary work the dose range in which the kinetics of lignocaine and tocainide showed first order kinetics was established. Doses within this range were chosen for the study of the effects of alterations of liver blood flow and liver enzyme activity on the kinetics of both drugs. Two enzyme inducing agents were used, 3,4 benzpyrene which induces hepatic enzymes without altering liver blood flow and phenobarbitone which induces hepatic enzymes and increases hepatic blood flow. Sotalol, a β-adrenoceptor antagonist was found to decrease cardiac output and liver blood flow. Liver blood flow was measured by the radioactively labelled microsphere method. By this method flow to other organs and fractional distribution of cardiac output can also be observed.

Changes in kinetics of lignocaine and tocainide at selected doses after intravenous and oral administration were observed in rats treated with either, 3,4 benzpyrene, phenobarbitone or sotalol.

The results were compared with those obtained from the two mathematical models, the "well-stirred" and the "parallel tube" models which predict change in kinetics when these major determinants of hepatic drug elimination, blood flow and metabolism are changed.

For tocainide, a metabolism dependent drug, both models gave a satisfactory prediction of changes in hepatic extraction ratio, systemic availability and AUC when blood flow and metabolism are altered.

Reasonably close prediction of kinetic changes of lignocaine a high hepatic clearance drug is obtained when hepatic enzymes are induced. For changes in liver blood flow with lignocaine the predictions for hepatic extraction ratio and $AUC_{i.v.}$ are close to the observed values but those for $AUC_{p.o.}$ were not with both models.

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# CHAPTER I

# INTRODUCTION

# CHAPTER I

## 1.1 Lignocaine

### 1.1.1 Historical aspects

Lignocaine was initially synthesized as a potential local anaesthetic agent in 1943 by Lofgren in Sweden. It was shown by Ehrenberg (1948) to be 3.3 times as active as procaine, the principal local anaesthetic used at that time and to have a more rapid onset of action. Lignocaine has subsequently become one of the most widely used drugs for local anaesthesia in medicine.

The antiarrhythmic properties of lignocaine were first recognised when it was used in resuscitating a patient with ventricular fibrillation after all other attempts had failed (Southworth, McKusick, Pierce and Rawson, 1950). Lignocaine was later used in the operating theatre by anaesthetists for this purpose, and in due course its action of preventing ventricular arrhythmias after acute myocardial infarction in animals was recognised (Carden and Steinhaus, 1956; Harris, Aquirre, Liptak and Brigham, 1955/56). In 1963 Harrison, Sprouse and Morrow reported the successful use of lignocaine given intravenously for cardiac arrhythmias that occur during and after open heart surgery. Lignocaine has subsequently become a drug of first choice for the prevention and treatment of ventricular arrhythmias especially in patients with acute myocardial infarction (Gianelly, von der Groeben, Spivak and Harrison, 1967).

A potential role for lignocaine as an antiarrhythmic drug stimulated the first pharmacokinetic studies. The physiological disposition of lignocaine compared with procaine in rats was studied by Sung and Truant (1954) using a spectrophotometric method for the determination of lignocaine. They demonstrated that lignocaine given intravenously disappeared very rapidly from the blood and found that

the drug was eliminated by metabolism mainly in the liver. Consistent with this observation Harrison, Sprouse and Morrow (1963) reported that lignocaine had a relatively short duration of antiarrhythmic effect when given to man as an intravenous bolus. Continuous intravenous infusion of lignocaine was reported to be effective in patients with acute myocardial infarction by Gianelly et al (1967). Following the development of gas chromatographic techniques which permitted greater sensitivity of measurement (Beckett, Boyes and Parker, 1965; Keenaghan, 1967; Rowland, Thomson, Guichard and Melmon, 1971) a more accurate and complete understanding of the pharmacokinetics of lignocaine became possbile (Boyes, Scott, Jebson, Godman and Julian, 1971; Tucker and Boas, 1971; Rowland, Thomson, Guichard and Melmon, 1971). Various workers were able to demonstrate that the dose of lignocaine could be related to antiarrhythmic effects (Jewitt, Kishon and Thomas, 1968; Gianelly et al, 1967) and a therapeutic concentration range became established (Gianelly et al, 1967; Grossman, Cooper and Frieden, 1969). These developments enabled successful dosage regimens for the attainment and maintenance of therapeutic blood lignocaine concentrations to be developed (Harrison and Alderman, 1972; Wagner, 1974; Aps, Jenkins, Poole-Wilson and Reynolds, 1975; Singh and Kocot, 1976; Greenblatt, Bolognini, Koch-Wesser and Harmatz, 1976; Sheridan, Rawlins, Crawford and Julian, 1977) and the drug is now regularly used in most coronary care units.

1.1.2 Chemical structure

Lignocaine (2-diethyl amino-2'-6'-acetoxylidide) is composed of three parts:

(i) a hydrophilic amino group (diethylamine);

(ii) a lipophilic aromatic group (2',6' xylidine);

(iii) an intermediate 2-carbon chain linking (i) and (ii) together containing an amide bond.

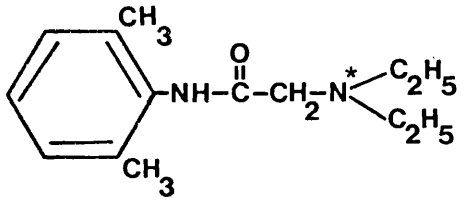
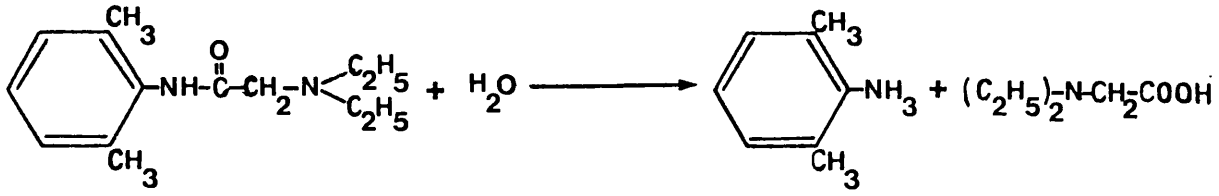


Figure 1.1 Chemical structure of lignocaine
MW(base) = 234.3
MW(HCl) = 270.8
N* is the tertiary amine

The amide linkage between the aromatic group and its connecting side chain is protected by two methyl groups attached to the aromatic ring in ortho-position. The stability of lignocaine to hydrolysis by acid or alkali is due to the presence of these ortho-methyl groups (Bullock and Grundy, 1955; Strother, Soong, Dev and Sadri, 1977), a general property known as the ortho-effect (Watson, 1949). Notwithstanding the basic stability of the lignocaine molecule, decomposition of the drug can occur in solution by hydrolysis of the amide bond (Bullock and Grundy, 1955) in the following manner:



1.1.3 Electrophysiological effects

In common with other drugs which have local anaesthetic action lignocaine prevents both the generation and the conduction of the impulse in peripheral nerve by blocking the transient increase in permeability of the nerve membrane to sodium ions. These effects are related to the action of lignocaine on the heart. Many investigators (Davis and Temte, 1969; Singh and Vaughan Williams, 1971; Mandel and Bigger, 1971) have attempted to define the electrophysiological properties of lignocaine on cardiac muscle and nerve and although controversy remains, there are a number of points of general agreement. Lignocaine in common with several other local anaesthetic antiarrhythmic agents has "membrane stabilising" effects. This results in decreased automaticity of pacemaker tissue, and fibrillation thresholds for ventricular muscle and Purkinje tissue are elevated in normal and in ischaemic hearts. The duration of the action potential in Purkinje tissue is decreased in contrast to quinidine which also has "membrane-stabilising" effects, and the effective refactory period (the time during which the fibre is unresponsive to stimuli) is shortened by lignocaine. In general terms , of the tissues which it effects, lignocaine, at therapeutic concentrations, has potent electrophysiological effects on the Purkinje system, less effect on the ventricular muscle and very little effect on the sinus and AV nodes and atrial muscle. The antiarrhythmic effects of lignocaine are probably due to reduced automaticity of pacemaker tissue and to reduced responsiveness of cardiac cells to excitation. The effect of lignocaine in shortening the action potential duration and the effective refractory period may also be beneficial since both become more uniform throughout the conducting system.

1.1.4 Haemodynamic effects

There are several reports of the haemodynamic effects of lignocaine in large laboratory animals but there is little data on small animals, for example the rat, which was used in the present work. Kao and Jalar (1959) found in the dog that lignocaine in a dose of 1 to 2 mg/kg body weight increased cardiac output by raising both heart rate and stroke volume and suggested that this was due to a central action. Constantino, Crockett and Vasko (1967) reported that intravenous doses of 2,4 and 8 mg/kg of lignocaine produced temporary decreases in cardiac output, systemic arterial pressure and stroke volume in anaesthetized dog. The effects in concious dogs were of lesser magnitude and of shorter duration. Other studies in dogs (Austen and Moran, 1965) showed a dose-dependent decrease in contractile force, heart rate and aortic blood pressure. Harrison and Alderman (1972) studied haemodynamic effects of lignocaine in dogs with induced acute myocardial infarction and found that infusions of lignocaine in doses of up to 200 µg/kg/min produced minimal circulatory change. On the other hand, bolus injection of 5 mg/kg lignocaine significantly decreased heart rate, arterial pressure and cardiac output. Benowitz, Forsyth, Melmon and Rowland (1974) studying lignocaine in monkeys found a 10 mg bolus, followed by an intravenous infusion of 100 µg per kg per minute for 2 or 3 hours caused no significant change in any systemic circulatory parameter; during infusion of lignocaine after loss of 30% of circulating volume by haemorrage, blood pressure and cardiac output remained stable, but heart rate and haematocrit decreased and stroke volume increased as intravascular volume expanded. Several investigators have reported that in normal humans lignocaine at therapeutic doses either by bolus or by intravenous infusion caused either no change or only minimal change in heart rate, cardiac output,

stroke volume, mean systemic and pulmonary artery pressure or peripheral arterial resistance and similar absence of haemodynamic upset is found in patients with chronic cardiac disease and with acute myocardial infarction (Stannard, Sloman, and Sangster, 1968; Jewitt, et al, 1968; Schumacher, Lieberson, Childress and Williams, 1968; Binnion, Murtagh, Pollock and Fletcher, 1969; Cullhed, 1969; Grossman et al, 1969). Although there are some reports (Harrision et al 1963; Harrison and Alderman, 1972) that lignocaine produces a decrease in ventricular contractile force, this depression is mild and of short duration. Overdosage of lignocaine can cause hypotension, probably due to a direct depressant effect on myocardial contractility, especially in a damaged heart (Harrison et al, 1963; Harrison and Alderman, 1972).

### 1.1.5 Lignocaine and cardiac arrhythmias

The principal use of lignocaine now is in the suppression of ventricular arrhythmias especially those complicating myocardial infarction. It is widely recognised that in acute cardiac injury due to ischaemia, death is often due to ventricular arrhythmia leading to ventricular fibrillation and circulatory failure. The importance of this field is such that it has been responsible for the establishment in most district hospitals of coronary care units which are largely geared to the management of cardiac arrhythmias. It says much for the efficacy and safety of lignocaine that of the many agents which have been used to suppress cardiac irregularity after myocardial infarction, it has been for several years and remains, for this condition, a drug of first choice (Lown, Fakhro, Hood and Thorn, 1967; Grossman, Lubow, Freiden and Rubin, 1968; Harrison and Alderman, 1972). Indeed it has been advocated that lignocaine should be used routinely in the

prophylaxis of myocardial infarction (Sheridan et al, 1977) uncomplicated by arrhythmia, although infact this is not a usual practice.

Arrhythmias emanating from atrial or nodal tissue are normally less sensitive to lignocaine.

### 1.1.6 Toxicity

Toxicity data are available for lignocaine in small and in large laboratory animals and in man. In mice, Smith and Duce (1971) found that large doses caused death with convulsions and calculated the $LD_{50}$ to be 292 mg/kg. The same authors found that doses of 10 mg/kg produced emesis in dogs. Boyes, Adam and Duce (1970) also found that large doses of lignocaine administered both i.v. and p.o. to dogs caused emesis at a time considerably after the maximum blood levels of the drug had been attained, an effect possibly due to metabolites of lignocaine. The most common side effects of lignocaine in man involve the central nervous system (Gianelly et al, 1967). Milder signs include dizziness, drowsiness, parethesiae, disorientation, agitation, twitching, double vision and diminished hearing. Seizures and respiratory arrest are among the more severe side effects and are encountered at blood lignocaine concentrations in excess of 9 µg/ml. Two of the major metabolites of lignocaine, monoethylglycylxylidide (MEGX) and glycylxylidide (GX) which are capable of inducing convulsions in mice and in dogs (Smith and Duce, 1971; Blumer, Strong and Atkinson, 1973) and central nervous system toxicity in man during conditions of prolonged constant intravenous infusion (Strong, Parker and Atkinson, 1973), may also be due to accumulation of these products of biotransformation. Adverse electrophysiological effects of lignocaine on the heart are uncommon but when present may be serious and take the form of sinus arrest, complete A.V. block and atrial or

ventricular dysrhythmias. (Deacock and Simpson, 1964; Pfeifer, Greenblatt and Koch-Weser, 1976; Benowitz and Meister, 1978). It is a general experience that adverse reactions occur more frequently in elderly patients and in patients with acute myocardial infarction and congestive cardiac failure, that is, in circumstances in which cardiac output and in turn liver blood flow are impaired (Zito, Reid and Longstreth, 1977).

### 1.1.7 Protein binding and tissue distribution

Various species differences have been reported in the distribution of lignocaine to the different elements of the blood. In rat, lignocaine was reported by Katz (1968) to distribute equally to red blood cells and to plasma and hence for that animal whole blood and plasma concentrations of lignocaine should be equivalent. At therapeutic blood concentrations in man (1.2 - 5.0 µg/ml) and in monkey 60-70% of lignocaine is bound to plasma proteins (Tucker, Boyes, Bridenbaugh, and Moore, 1970; Difazio, 1975; Benowitz, Forsyth, Melmon and Rowland, 1974a). At high concentrations, binding decreases (Tucker et al, 1970). Lignocaine is less well bound to human erythocytes than to plasma protein and in consequence for any given blood sample the concentration of lignocaine in the plasma is higher than the value in whole blood. Tucker et al (1970) found that at a whole blood lignocaine concentration of 0.5 µg/ml the plasma: blood ratio was 1.5. The initial distribution of lignocaine after entering the blood stream is highly dependent on regional blood flow. One minute after intravenous administration of a $^{14}C$ labelled bolus dose to rat, the highly perfused tissues, i.e., heart, lung, liver, brain, kidney and spleen were found to contain 70% of the radioactivity (Katz, 1968). Lignocaine has a $pK_a$ of 7.85 and at pH 7.4, the ratio of ionized to unionized drug is 3:1. However, the molecule

as a whole is highly lipid soluble and the drug partitions extensively into body tissues. In rat, 30 min after lignocaine had been administered intramuscularly at a dose of 10 mg/kg, Akerman, Ström, Ross and Telc (1966) found high concentrations in kidney, lung, spleen, brain, heart and liver. Similar findings have been in dog by Ahmad and Medzihradsky (1971) and in rhesus monkey by Benowitz et al, (1974b). During steady state infusion in man Benowitz and Meister (1978) estimated that only about 6% of the amount of lignocaine in the body was present in the circulating volume. Although the rate of accumulation of lignocaine in various tissues is to a considerable extent dependent on the fraction of the cardiac output which is received, the well-perfused tissues such as heart, brain, kidney and other viscera do not contain the major body load of administered lignocaine. Thus muscle and adipose tissue which are less well perfused but which are larger in mass, in fact become the major storage reservoir of lignocaine.

### 1.1.8 Pharmacokinetics

The isolated perfused rat liver and the perfused rat liver *in situ* have frequently been used to study lignocaine elimination but detailed kinetic data for the rat as a whole animal, as used in the present study, have not been found in the literature. After intravenous injection to the rhesus monkey, Benowitz et al (1974a) found a very rapid early exponential decline in blood concentration with a half-life ($t_{\frac{1}{2}}$) of 1-2 min which was followed by a second exponential decline with a $t_{\frac{1}{2}}$ of about 15 min. A similar biphasic decline in blood concentration after an intravenous bolus dose was found in 10 normal volunteers by Rowland et al (1971) the initial rapid phase having a mean $t_{\frac{1}{2}}$ 6.8 min and the subsequent slower elimination phase having a mean $t_{\frac{1}{2}}$ of 108 min.

These and other authors (Boyes et al, 1970) found that the pharmakinetics of lignocaine could be described adequately by a two-compartment open system. Rowland et al (1971) also noted that the volume of distribution of the central compartment which describes the initial distribution space and the steady state distribution volume which is an indication of the total amount of lignocaine in the body as a function of the blood lignocaine concentration during constant intravenous infusion of the drug were both in excess of the blood volume and of the body water spaces respectively which implies considerable extravascular distribution of lignocaine.

The liver is the major site of elimination of lignocaine (Sung and Truant, 1954; Hollunger, 1960a and b) and in all species studied extensive extraction of the drug has been shown to occur in a single passage through the liver. The highest extraction ratio (E) is found in rat (0.99) (Pang and Rowland, 1977), other approximate values for E being: man, 0.7 (Boyes et al, 1970; Perucca and Richens, 1979, Bennett, Aarons, Bending, Steiner and Rowland, in press); dog, 0.78 (Branch, Shand, Wlikinson and Nies, 1973) and monkey, 0.66 (Benowitz et al, 1974a). By contrast, in cat, Lautt and Skelton (1976) found much lower hepatic extraction (E = 0.28). In consequence of its high heptic clearance lignocaine is an example of a drug which shows "first pass" effect i.e. extensive presystemic elimination. Availability of the drug to the systemic circulation by the oral route has been shown to be greatly reduced in man (Boyes et al, 1971) and in dog (Boyes, Adams and Duce, 1970). A feature of this type of drug, which is discussed more fully later, is that the rate of elimination is very dependent on the rate of delivery of drug to the liver, that is, on liver blood flow. Total hepatic blood flow in most species is approximately 25% of cardiac output and of this about 25-30% is derived from the hepatic artery, the remainder being supplied by the portal vein. Thus changes

in cardiac output or in the regional distribution of the output may cause changes in the blood supply to that organ and may thereby be expected to affect the elimination of lignocaine. In patients with myocardial infarction who had cardiac failure, the terminal half-lives of lignocaine were longer than those in myocardial infarction patients without cardiac failure (Prescott, Adjepon-Yamoah and Talbot, 1976; Aps. et al, 1976) a finding which is consistent with the dependence of lignocaine blood concentration on cardiac output and hepatic blood flow. Benowitz et al (1974) found reduced clearance and increased half-life of lignocaine in rhesus monkeys subjected to haemorrhage of 30% of circulating volume. In the same study the haemodynamic changes produced by intravenous infusion of isoprenaline and of noradrenaline produced marked alteration in steady state blood lignocaine concentration.

Drugs may affect the pharmacokinetics of lignocaine by other mechanisms. Perucca and Richens (1979) found that the systemic availability of lignocaine administered to epileptic patients on anticonvulsant drugs was much less than that found in normal subjects. This finding is almost certainly due to induction of hepatic microsomal enzymes by anticonvulsant drugs and its implication are examined in the later sections of this work.

### 1.1.9 Metabolism and excretion

Lignocaine is almost entirely metabolised by the liver (Sung and Truant, 1954; Hollunger, 1960a and b), and less than 5-10% is excreted unchanged in the urine (Eriksson, Granberg and Ortengren, 1966). The metabolism of lignocaine *in vitro* was originally established by Hollunger (1960a and b) using rabbit liver. He demonstrated that lignocaine was metabolised in an hepatic microsomal enzyme system by oxidative N-deethylation which required both oxygen and reduced NADPH in the reaction.

Oxidative biotransformation of lignocaine also involves the flavoprotein cytochrome c reductase (Williams and Kamin, 1962) and the haemoprotein cytochrome P-450 (Omura, Sato, Cooper, Rosenthal and Estabrook, 1965), the term P-450 referring to the ability of the reduced form of the haemoprotein to react with carbon monoxide to yield a complex which has its main absorption peak at 450 nm (Omura and Sato, 1964). Similar requirements have been shown for the metabolism of several other drugs (Brodie, Gillette and La Du, 1958; Gillette, 1963). The reactions may be expressed in the following general form:

1. $NADPH + A + H^+ \xrightarrow[\text{reductase}]{\text{NADPH cytochrome c}} AH_2 + NADP^+$

2. $AH_2 + O_2 \longrightarrow$ "active oxygen complex"

3. "active oxygen complex" + drug substrate $\longrightarrow$ oxidized drug + A + $H_2O$

---

$NADPH + O_2 + \text{drug substrate} + H^+ \longrightarrow NADP^+ + \text{Oxidized drug} + H_2O$

where A is the oxidized form and $AH_2$ is the reduced form of cytochrome P-450. One molecule of $O_2$ is consumed for each molecule of substrate oxidized, one atom of O being introduced into the substrate, the other being reduced to form $H_2O$. The metabolising system is thus called a mono-oxygenase or mixed function oxidase system. Pretreatment with certain drugs, e.g. 3,4-benzpyrene or phenobarbitone, greatly increases the metabolising capacity of this enzyme system.

Lignocaine has been found to bind with high affinity to microsomal cytochrome P-450 (Moldeus, Grudin, von Bahr and Orrenius, 1973) and at high concentration was noted to be unusally rapidly deethylated by the microsomes with a $V_{max}$ of about 15 nmole/mg protein, min and $K_m$ of 250 µM by Nyberg, Karlen, Hedlund, Grudin and von Bahr (1977). They proposed that the high affinity of lignocaine for the cytochrome P-450

system as well as the rapid oxidation of the drug are important determinants of the extensive liver extraction and first pass elimination of the drug. Von Bahr, Hedlund, Karlen, Bäckström and Grasdalen (1977) reported that there are two binding sites for lignocaine to microsomal cytochrome P-450, a "low affinity site" catalysing deethylation and a "high affinity site" catalysing aromatic hydroxylation. This may explain the larger proportion of aromatically hydroxylated as opposed to deethylated metabolite of lignocaine which is normally found. Thus Keenaghan and Boyes (1972) reported that 65% of an oral dose (25 mg/kg) of lignocaine given to female rats, is recovered in urine as 3-hydroxylated metabolites, and only 0.7% as MEGX (monoethylglyeinexylidide, a deethylated product).

Hepatic metabolism of lignocaine has been studied extensively in animals (Keenaghan and Boyes, 1972; Hollunger, 1960a and B; Akerman, et al, 1966; Di Fazio and Brown, 1972) and man (Beckett, Boyes and Appleton, 1966; Mather and Thomas, 1972; Adjepon Yamoah and Prescott, 1973; Breck and Trager, 1971; Heinonen, Takki and Jarho, 1970; Stenson, Constantino and Harrison, 1969). Keenaghan and Boyes (1972) observed in man that 73% of an administered lignocaine dose (Table 1.1) appeared in the urine as 4-hydroxy xylidine, while only minor quantities of other metabolites appeared. Urinary excretion in the dog consisted mainly of 4-hydroxyxylidine and significant quantities of glycylxylidide (GX). In contrast, the rat excreted mainly 3-hydroxy lignocaine and 3-hydroxy MEGX, with less 4-hydroxyxylidine. Figure 1.2 shows the structures of lignocaine and its metabolites. The arrows in the figure represent the probable major pathways of metabolism. Keenaghan and Boyes (1972) compared the metabolic products of lignocaine in rat, guinea-pig, dog and man, and the proportion of each metabolite excreted by the various species are summarized in Table 1.1. It can

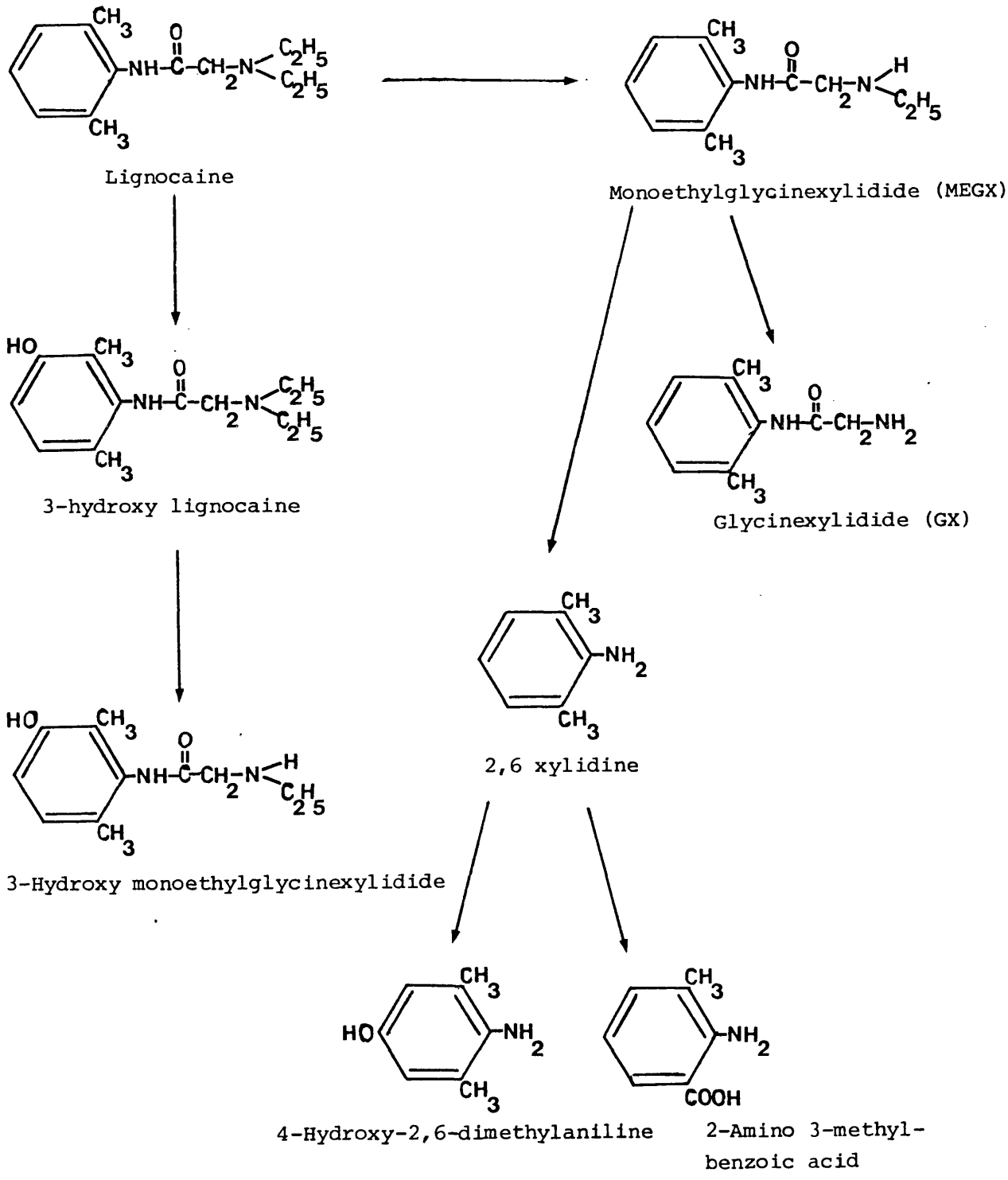



Figure 1.2 Summary of known pathways of metabolism of lignocaine

be seen that inter-species variability in the excretion of lignocaine metabolites does exist. Nevertheless the species listed all appear to produce the same range of metabolic products suggesting that differences between species are more those of the proportion of drug metabolised by different routes rather than the routes themselves. Furthermore it is a feature common to all species studied that hepatic extraction and biotransformation of lignocaine is rapidly achieved, thus species differences in metabolism are unlikely to be a major obstruction to the extrapolation of animal data, and in the case of the present work, rat data, to the findings in man.

Biliary excretion of lignocaine is a minor factor in elimination. Katz (1968) found that less than 3% of $^{14}C$-labelled lignocaine dose administered to a dog appeared in the bile after five hours. Keenaghan and Boyes (1972) reported about 30% recovery of radioactivity in the bile collected for 24 hr in rats given $^{3}H$ lignocaine either p.o. or i.v. This radioactivity was exclusively from metabolites, no unchanged lignocaine being found in the bile. Metabolism by other tissues is minimal.

Lignocaine, a weak base ($pK_a$ 7.85) is reabsorbed from the kidney tubules by nonionic diffusion and its renal clearance is affected by changes in urinary pH but not significantly by urine flow rate (Eriksson and Granberg, 1965). However, excretion of unchanged lignocaine by the kidney is a minor route of elimination. About 2.8% of the dose administered was reported to be normally excreted unchanged in the urine in rats (Keenaghan and Boyes, 1972) and about 3-11% in man (Sung and Truant, 1954; Beckett et al, 1966).

Table 1.1*: Species variation in the metabolism of lignocaine

| Compound | Percentage of dose recovered in 48hr urine | | | |
|---|---|---|---|---|
| | Rat | Guinea-pig | Dog | Man |
| Lignocaine | 0.2 | 0.5 | 2.0 | 2.8 |
| Monoethylglycinexylidide | 0.7 | 14.9 | 2.3 | 3.7 |
| Glycinexylidide | 2.1 | 3.3 | 12.6 | 2.3 |
| 3-Hydroxylignocaine | 31.2 | 0.5 | 6.7 | 1.1 |
| 3-Hydroxymomonethyl-glycinexylidide | 36.9 | 2.0 | 3.1 | 0.3 |
| 2,6-Xylidine | 1.5 | 16.2 | 1.6 | 1.0 |
| 4-Hydroxy-2,6-di-methylaniline | 12.4 | 16.4 | 35.2 | 72.6 |

* from Keenaghan and Boyes (1972)

1.1.10 Metabolites of lignocaine

The principal metabolites of lignocaine are MEGX and GX. MEGX has been noted to cause convulsions in mouse, rat and dog (Smith and Duce, 1971; Blumer et al, 1973). The convulsant activity of MEGX has been estimated to be equivalent to that of lignocaine (Blumer et al, 1973). GX may cause death in animals before convulsions are seen and it is capable of potentiating the convulsant activities of MEGX and lignocaine (Blumer et al, 1973).

Both MEGX and GX may be found in significant concentrations in the blood of patients receiving lignocaine therapeutically (Adjepon-Yamoah and Prescott, 1973; Collinsworth, Strong, Atkinson, Winkle, Perlroth and Harrison, 1975; Halkin, Meffin, Melmon and Rowland, 1975; Strong, Mayfield, Atkinson, Burris, Raymon and Webster 1975; Prescott et al, 1976). Both metabolites have antiarrhythmic effects in animal and in man. However, MEGX, the first de-ethylation product exhibits 83% of the anti-arrhythmic activity of lignocaine (as measured by its ability to suppress digitalis-induced arrhythmias in guinea-pig atrium) and it is also a local anaesthetic, whereas in common with other primary amines, GX has poor local anaesthetic properties and exhibits only about 10% of lignocaine's anti-arrhythmic effect (Strong et al, 1973; Burney, Di Fazio, Peach, Petrie and Silvester, 1974). After oral administration of lignocaine to normal volunteers, Bennett et al (in press) found that blood concentrations of MEGX and of lignocaine were similar, indicating that the oral availability of lignocaine based on anti-arrhythmic activity would be greater than the estimate calculated from measurements of lignocaine concentration alone. In the study quoted the estimated half-lives of MEGX in three normal subjects ranged between 28 and 94 min which are values comparable to those reported by Adjepon-Yamoah

and Prescott (1973). Similar estimates of half-life were made in three patients who received therapeutic infusions of lignocaine intravenously by Halkin et al (1975). It is probable that in some patients who receive lignocaine by intravenous infusion, appreciable accumulation of MEGX contributes to anti-arrhythmic and possibly also to toxic effects (Strong et al, 1973; Halkin et al, 1975; Prescott et al, 1976; Nation, Triggs and Selig, 1976).

## 1.2 Tocainide

### 1.2.1 History and chemistry

Tocainide (2-amino-2',6' propionoxylidide HCl), a primary amine analogue of lignocaine was synthesised with the object of developing an anti-arrhythmic drug that is less rapidly eliminated than lignocaine. In common with lignocaine, tocainide has two ortho-methyl groups on the benzene ring, which confer considerable stability to the molecule, the phenomenon of "ortho-effect".

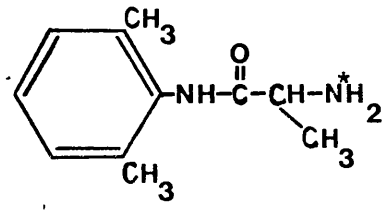


Figure 1.3 Chemical structure of tocainide

MW (base)= 192.26

MW (HCl) = 228.72

N* = the primary nitrogen

In early work tocainide was found to have similar electrophysiological properties to lignocaine and it was shown to be effective in suppressing ventricular arrhythmias after experimental myocardial infarction in dog (Coltart, Berndt, Kernoff and Harrison, 1974). Evidence of its low hepatic extraction in dog (Coltart et al, 1974) and in man, together with high bioavailability (Lalka, Melvin, Meyer, Duce and Elvin, 1976) led to its introduction in to clinical use. In 1976 McDevitt, Nies, Wilkinson, Smith, Woosley and Oates (1976) reported that oral doses of tocainide given to patients with frequent premature ventricular contraction were successful in suppressing that arrhythmia.

### 1.2.2 Electrophysiologic effects

Data in the literature on the electrophysiological effects of tocainide are scant and the following account is based on unpublished data supplied by Astra Pharmaceutical Products Inc. Tocainide appears to resemble lignocaine in several of its effects. When applied to desheathed isolated frog sciatic nerve tocainide has a local anaesthetic acition similar to that of lignocaine. In a voltage-clamped isolated frog nerve preparation, in which an approximate 80% block of sodium current is needed to block conduction, tocainide appeared to be about one third as effective as lignocaine. In the isolated dog Purkinje-papillary muscle preparation, tocaininde shortened the effective refractory period and action potential duration in single Purkinje fibres. Tocainide may be classed as an anti-arrhythmic agent with "membrane stabilising" properties and it bears a resemblance to both lignocaine and quinidine in its actions. (Investigator's brochure tocainide HCl, Astra Pharmaceutical Products Inc.).

### 1.2.3 Haemodynamic effects

Tocainide appears to have little haemodynamic activity. In concious dogs, Duce, Smith, Boyes and Byrnes (1973) reported that multiple oral dosing with tocainide (25 to 50 mg/kg every 4 hours) did not alter systemic arterial pressure or central venous pressure. Coltart et al, (1974) failed to find significant circulatory changes in dogs one hour after administration of 60 mg/kg orally but when the dose was increased to 120 mg/kg a significant decrease in heart rate to 24-32% of control value was observed.

Administered to normal volunteers in doses of 10 mg to 1000 mg, tocainide caused no significant change in blood pressure, heart rate or systolic time intervals. (Investigator's Brochure Tocainide HCL, Astra Pharmaceutical Ltd., 1977). The electrocardiographic parameters of PR interval, QT interval and QRS duration were also unchanged.

McDevitt et al (1976), in a study of patients with frequent premature ventricular contractions (PVCs) gave single oral doses of tocainide ranging between 10 mg and 1200 mg in an incremental sequence. No significant effects on arterial blood pressure, on heart rate or on the electrocardiogram of normally conducted beats were observed. More recently, Schwartz, Covino, Duce, Narang, Fiore, Markelis, Rizvi and Smith, (1979) reported that following intravenous infusion of tocainide to cardiac patients, left ventricular pressure was significantly reduced although other parameters of cardiac function (i.e., heart rate, cardiac output, cardiac index, stroke volume, systemic vascular resistance and pulmonary vascular resistance) were unaltered. It was suggested that this reflects a direct depressant effect of tocainide on myocardial contractility in these patients.

### 1.2.4 Anti-arrhythmic effects

Tocainide has been found to be an effective anti-arrhythmic drug when studied in standard laboratory models of cardiac arrhythmia. Moore and Spear (1973) reported that chloroform-induced ventricular fibrillation in mice was diminshed. They also observed that in dog, the threshold for ventricular fibrillation induced by electrical impulses was raised by the administration of tocainide. In the same study tocainide slowed the rate of discharge of atrial pacemakers in dog and completely suppressed ventricular pacemakers with induced complete atrio -ventricular block. Similar studies by Duce, Smith, Boyes and Byrnes (1973) confirmed the effects of tocainide on chloroform-induced ventricular fibrillation in mice. These workers also found a dose-related suppression of induced ventricular ectopic beats and of arrhythmias caused by ouabain in dog. These effects of tocainide occurred at plasma concentrations of 15-30 µg/ml in which range no

change in haemodynamic parameters such as cardiac output, blood pressure and heart rate were noted. Coltart et al (1974) confirmed that at these concentrations of tocainide, suppression of ventricular ectopic beats occurs in dog.

Anti-arrhythmic effects of tocainide have also been demonstrated in man. McDevitt et al (1976) in their study of patients with frequent PVCs found that oral doses of tocainide in excess of 100 mg were associated with a 60% reduction in PVCs in the 5 hours after a dose. At oral doses of 400-600 mg, Winkle et al (1976) reported a 90% reduction in PVCs with tocainide (plasma concentrations of 5-10 µg/ml). Winkle et al (1976) found a 70% reduction in PVCs at plasma concentrations of tocainide in excess of 6 µg/ml.

### 1.2.5 Toxicity and side effects

In animal toxicity testing, deaths in rodents due to tocainide were associated with convulsions (Adam, 1972; Smith, 1972) and in dogs and monkeys (Duce, 1972) sub-lethal doses of tocainide caused emesis and prolonged, severe seizures. Cumulative oral doses of tocainide given to dogs with induced ventricular arrhythmias also produced emesis and convulsions (Duce, 1972) but these effects occurred after the arrhythmia had been abolished. Subacute toxic effects in animals include ataxia and sedation (Duce, 1972).

The common adverse effects of tocainide in patients are related to the gastrointestinal tract (anorexia and nausea) and to the central nervous system. Schwartz et al (1979) reported that at blood levels of 9-29 µg/ml, which are largely but not entirely in excess of the therapeutic concentrations, tocainide caused lightheadedness, tremor, disorientation and confusion.

1.2.6 Pharmacokinetics

After intravenous injection Schwartz et al (1979) noted that in man, blood concentrations of the drug fell rapidly for 15 mins and then declined more slowly, that is the disappearance curve of tocainide from the blood exhibited a bi-exponential form. Lalka et al (1976) found that blood concentration data after intravenous injection of tocainide could be fitted satisfactorily to a two-compartment open pharmacokinetic model. However, the initial exponential decline is of short duration, that is the ratio $A/\alpha$ is very small compared to the ratio $B/\beta$ (see Chapter 1.3.; Basic Pharmacokinetics) and for many purposes the pharmacokinetics of tocainide may be described adequately by a one compartment open model. Most available kinetic data refer to man. The average disposition half-life ($t_{\frac{1}{2}}\ \beta$) for this drug in man was found by Lalka et al (1976) to be 11.3 hr. In four normal males the mean initial dilution space, $V_p$ (0.91 L/kg) and the volume of distribution at steady state, $V_{d_{ss}}$, (1.44 L/kg) were reported to be greater than the human blood volume and body water spaces respectively implying that there is extravascular concentration of tocainide in a manner similar to that of lignocaine. Clearance of tocainide from the blood is reported in man to be 62% hepatic and 38% renal. Little comparable data exists in other species.

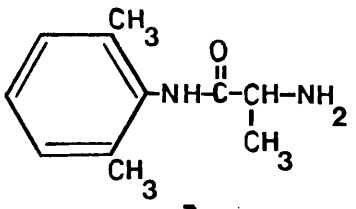
Binding of tocainide to plasma protein was studied by Lalka et al (1976) who found that in the clinically effective range of 2-13 μg/ml approximately 50% of the drug is bound, that is the plasma: blood ratio is approximately unity. These workers also reported that there are two classes of binding site of tocainide to human plasma, one with low capacity and high affinity and another with low affinity and high capacity.

After oral administration to laboratory animals and to man, tocainide is well absorbed from the gastrointestinal tract (Duce et al, 1973; McDevitt et al, 1976) and peak blood concentration occurs approximately 60 min after dosing. Lalka et al (1976) found that the rate of absorption of tocainide was so rapid that it was not possible to assign an order to the process. Bioavailability of tocainide has been found to approach 100% whether calculated by excretion of parent drug and metabolites in urine (Lalka and Feldman, 1974) or by direct comparison of areas under blood concentration-time curves following intravenous and oral dosing (Lalka et al, 1976). It has also been recorded that the presence of food in the stomach may reduce peak blood concentrations by up to one third but bioavailability is not altered by food (Lalka et al, 1976).

### 1.2.7 Metabolism and excretion

The complete biotransformation pathways and individual metabolic products of tocainide in various species have yet to be elucidated. In microsomal incubation experiments (Nyberg et al, 1977) tocainide, in contrast to lignocaine, was found to have a very low affinity for liver microsomal cytochrome P-450.. The primary amine structure of tocainide which resists rapid N-deethylation together with its low affinity for the principal drug metabolising enzyme system are compatible with the slower rate of hepatic metabolism of tocainide as compared with lignocaine.

The principal metabolites of tocainide which has been proposed (Investigator's Brochure Tocainide HCl, Astra Pharmaceutical Ltd., 1977; Elvin, Keenaghan, Byrnes, Tenthorey, McMaster, Takman, Lalka, Maninon, Baer, Wolshin, Meyer and Ronfeld, 1980) are shown in Figure 1.4.

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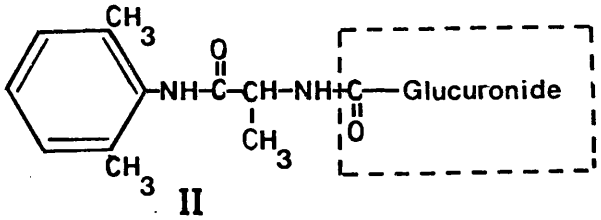


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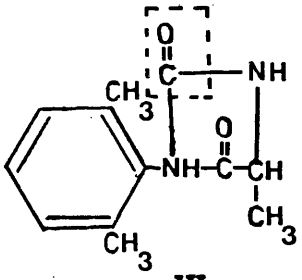


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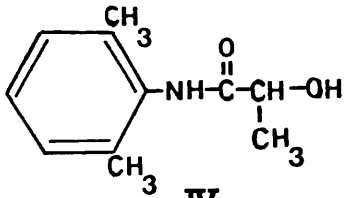


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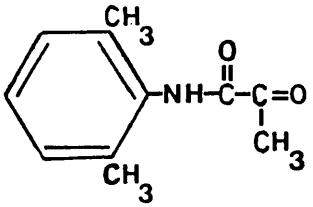



V

Figure 1.4 Tocainide and known or suspected products of elimination

About 25% of the dose is accounted for by the Compound III which is believed to be a metabonate, that is, a substance formed after excretion from the body. Compound III is believed to derive from the substance labelled II which is an hypothesised metabolite in which a carbonyl group has been added between the tocainide and the glucuronic acid molecules. Only small amounts of metabolites IV and V are present in urine and about 30% of an administered dose has yet to be accounted for.

## 1.3 Basic pharmacokinetic concepts

Dynamic processes feature in almost all facets of pharmacology. Pharmacokinetics has been defined by Notari (1975) as that branch of pharmacology which is concerned with "the study of the kinetics of absorption, distribution, metabolism and excretion of drugs and their pharmacologic, therapeutic or toxic responses in animals and man". The objectives of pharmacokinetics include the derivation of mathematical expressions and the determination of constants which can be used to depict and to predict the time-course of drug concentration and effect in the body. The temporal relationships of drug effect depend on the dosage regimen used and on the absorption, disposition and elimination processes which in turn affect drug concentration at its site of action. A problem in defining accurate dosage regimens or in acheiving a meaningful understanding of biological responses is that drug at its site of action is usually inaccessible to measurement. In practice, most often drug measurements are made in blood or plasma and expressed graphically in the form of a concentration-time profile which can be analysed mathematically.

### 1.3.1 Compartmental analysis

The most common approach to the pharmacokinetic characterization of a drug is to express the body as a system of compartments, even though these compartments may not specifically correspond to well-defined anatomical or physiological spaces or volumes. Rather compartments are mathematical concepts postulated to explain observed drug concentrations on the basis of the amount of the drug in the body. In particular circumstances a compartment may represent a specific anatomical entity (e.g. G.I. tract or liver), a specific physiological entity (e.g. blood or urine) or a non-specific collection of entities

(e.g. poorly or well-perfused tissues). Drug transfer from one compartment to another is usually assumed to be a first order process, that is, the rate of exit of drug from a given space is proportional to the concentration of drug within it. In the presence of capacity limited elimination or of saturation of a transport system, the processes become zero order. Appropriate rate constants are thus assigned to different processes.

The one-compartment open model

If after intravenous injection, a drug distributes instantaneously and homogenously to the tissues and fluids of the body, and if drug elimination from the body is a first order process then the relationship between the logarithm of the concentration of drug in the blood and the time after injection is linear as shown in Figure 1.5 and the body

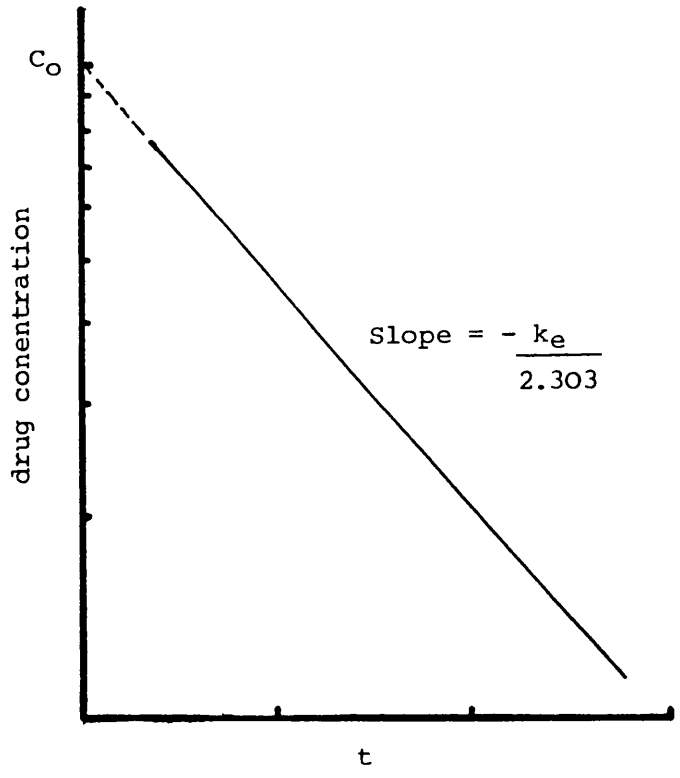



Figure 1.5 Schematic graph of drug concentration in the blood plotted on a logarithmic scale against time after a single intravenous bolus of a drug when the body is depicted as an open one compartment linear system

The half-life ($t_{\frac{1}{2}}$) of the drug in the blood is the time for any given concentration to decrease by one half. In the case of a drug which is eliminated in a manner characterised by first order kinetics, $t_{\frac{1}{2}}$ is independent of dose. It follows that if a series of different doses of a drug are given to the same individual, a plot of blood concentration/dose with respect to time will give a line which is independent of dose. This is known as the principle of superposition. Lengthening of half-life with increase of dose is an indication that elimination is no longer first order, that is that saturation of the elimination process is occurring.

A drug which distributes rapidly and is eliminated by first order kinetics such that its blood concentration-time profile is monophasic as in Figure 1.5 can be regarded as one which treats the body as a one-compartment open system. This is depicted in Figure 1.6

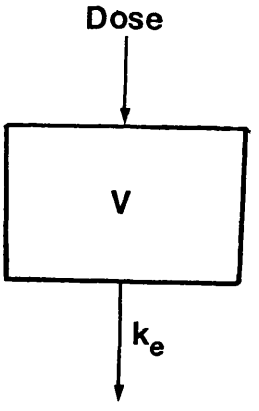



Figure 1.6 One-compartment open model

where V is the apparent volume of distribution of the drug in the body ($V_d$) and $k_e$ is the first order rate constant for overall elimination of drug from the body. It follows that for such a system there is a

proportionality between the concentration of drug in the compartment and the rate of elimination, that is

rate $\alpha$ concentration eq. (1)

Expressed in differential form

$$\frac{dc}{dt} = -k_e C \qquad \text{eq. (2)}$$

where

C is drug concentration

t is time

and $k_e$ is the first order rate constant for the elimination process with units of reciprocal time ($t^{-1}$).

If instantaneous input and distribution of drug are assumed, then the relationship between blood concentration in the system and time can be found by integration of eq. (2) to give

$$C_t = C_o e^{-k_e t} \qquad \text{eq. (3)}$$

where $c_t$ is the drug-concentration at time t and $c_o$ is the drug concentration at zero time, obtained by extrapolation of the $\log_{10}c$ versus t plot, and e is the base of the natural logarithm (ln). It follows that

$$\ln C = (\ln C_o) - k_e t \qquad \text{eq. (4)}$$

and from the relationship

$$2.303 \log_{10} C = \ln C \qquad \text{eq. (5)}$$

$$\log_{10} C = (\log_{10} C_o) - k_e t / 2.303 \qquad \text{eq. (6)}$$

Since eq. 6 is a simple linear equation the rate constant, $k_e$ may be obtained from the slope of the line. It may also be derived from $t_{\frac{1}{2}}$ according to the relationship

$$k_e = \frac{0.693}{t_{\frac{1}{2}}} \qquad \text{eq. (7)}$$

The two-compartment open model

In practice whether or not blood concentrations of a drug demonstrate one-compartment characteristics often depends on how soon the first blood samples are taken. In a survey of the pharmacokinetic characteristics of 38 drugs Dvorchik and Vessel (1978) found that considerable errors were associated with calculation of clearance on the basis of a one-compartment model if drug disposition followed multi-compartment kinetics, and that the magnitude of the error was related to the initial distribution of the particular drug. Generally after single intravenous injection blood concentrations of most drugs decrease in an initial rapid phase, after which there is a phase or phases of slower decline in blood concentration. A plot of the logarithm of blood concentration against time can often be resolved into two linear components. The method of residuals may be used to achieve this graphically, a process also called "feathering" in which differences in concentration between the extrapolation of the slower phase of the logarithm of concentration-time curve to zero time (B) and the observed blood concentration-time curve at various times are used to construct the initial linear component (Notari, 1975; Wagner, 1975), as demonstrated in Figure 1.7.

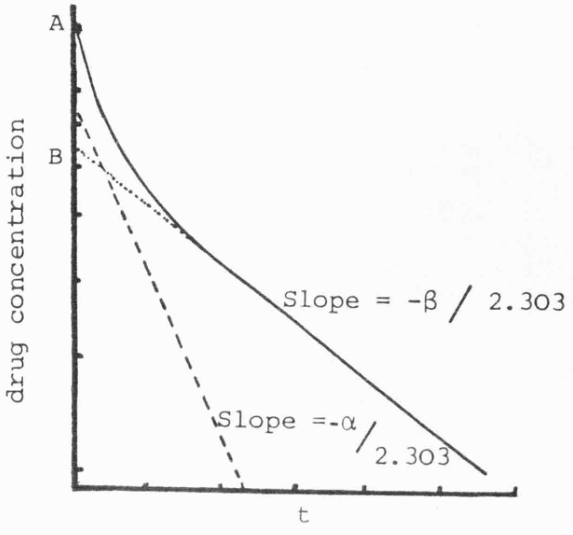



Figure 1.7 Semilogarithmic plot of blood drug concentration versus time after a single intravenous bolus of a drug when the body is depicted as an open two-compartment linear system

If the slopes of the rapid and slow exponential phases are designated $-\alpha/2.303$ and $-\beta/2.303$, respectively, where $\alpha$ and $\beta$ are apparent first order rate constants, and the intercepts on the concentration axis are A and B, then the entire blood concentration-time curve may be described by the following bi-exponential equation:

$$C_t = Ae^{-\alpha t} + Be^{-\beta t} \qquad \text{eq. (8)}$$

when decline in drug concentration in the blood is biexponential, it is justifible to depict the body as an open two-compartment system. The commonest model of this type conceives of a small volume central compartment and a large volume peripheral compartment. Drug is administered to and is elimination from the central compartment as is shown in Figure 1.8

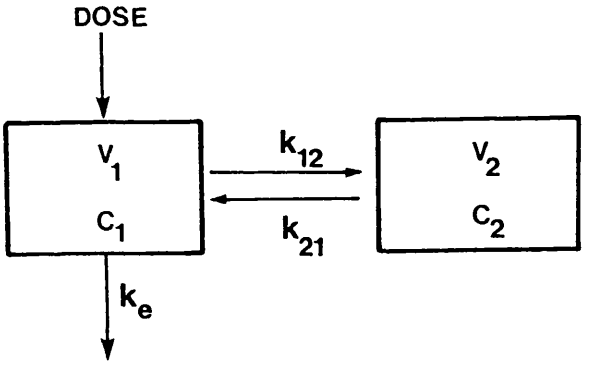



Figure 1.8 Two-compartment open model

$C_1$ and$_2$ represent drug concentration in the central and peripheral compartment respectively. Similarly $V_1$ and $V_2$ indicate the volumes of these compartment, $k_{12}$ and $k_{21}$ are the first order rate constants of drug transport between the central and peripheral compartments and $k_e$ is the first order rate constant for elimination of drug from the central compartment. Although these compartments are theoretical,

for many drugs, the blood and highly perfused tissues such as liver, kidneys, heart and lungs are probably representative of the central compartment. It is for this reason that elimination is depicted as occurring from that compartment.

Mayersohn and Gibaldi (1971) provided a mathematical solution to the two-compartment open model enabling the rate constants $k_{12}$, $k_{21}$ and $k_e$ to be determined in terms of $\alpha$, $\beta$, A and B as follows:

$$k_{21} = \frac{A\beta + B\alpha}{A + B} \qquad \text{eq. (9)}$$

$$k_e = \frac{\alpha\beta}{k_{21}} \qquad \text{eq. (10)}$$

$$k_{12} = \alpha + \beta - k_{21} - k_e \qquad \text{eq. (11)}$$

1.3.2. Apparent volume of distribution

Although in compartmental analysis the body is conceived of as consisting of one or more homogeneous spaces, it is obvious that in fact this not so. Drug concentrations may well be different in tissues such as muscle, liver, adipose tissue and blood. However, for many drugs the ratio of drug in the blood to that in the remainder of the body is reasonably constant. Thus it is possible to derive a proportionality constant which relates the blood concentration of drug (C) to the total amount of drug in the body (A);

$$A = Vd\ C$$

This proportionality constant is known as the apparent volume of distribution (Vd) and it can be derived in various ways. (Riegelman, Loo and Rowland, 1968; Benet and Ronfeld, 1969; Gibaldi, Nagashima and Levy, 1969; Perrier and Gibaldi, 1973).

Distribution volume by extrapolation

If the body is regarded as a single compartment then Vd can be derived from the intravenous dose which represents the amount of drug in the body at zero time and the blood concentration at that time, Co found by the extrapolation of the plot of logarithm of blood concentration with respect to time;

$$Vd = \frac{\text{i.v. dose}}{C_o} \qquad \text{eq. (12)}$$

However, the kinetic characteristics of many drugs are compatible with a two-compartment open system and with such a model the extrapolated value for B is given by

$$B = \frac{\text{dose } (k_{21}-\beta)}{V_1\ (\alpha-\beta)} \qquad \text{eq. (13)}$$

Where $V_1$ is the volume of the central compartment. This enables calculation of Vd by extrapolation ($Vd_{extrap}$)

$$V_d \text{ extrap} = \frac{\text{i.v. dose}}{B} = \frac{V_1\ (\alpha-\beta)}{k_{21}-\beta} \qquad \text{eq. (14)}$$

Distribution volume by area

The concept of a distribution volume implies an equilibrium state and this is a condition not met by extrapolation methods of calculation. Nagashima, Levy and O'Reilly (1968) drew attention to the fact that after bolus administration of a drug a "pseudo-distribution" equilibrium exists when the terminal phase of a logarithm of concentration versus time plot becomes linear. In other words the observed blood concentrations may be related to the total amount of drug in the body at all times after distribution equilibrium has been attained. For a drug which displays the characteristics of removal from the blood described by eq.(8), in time, the first exponential term $Ae^{-\alpha t}$ becomes

negligible i.e. the equation becomes monoexponential and accordingly

$$C_t = Be^{-\beta t} \qquad \text{eq. (15)}$$

Integration of eq. (8) between zero and infinity gives a value for the area under the concentration-time curve:

$$\text{area} = \int_0^{\alpha} Cdt = \frac{A}{\alpha} + \frac{B}{\beta} \qquad \text{eq. (16)}$$

Correspondingly eq. (15) becomes

$$\text{area} = \frac{B}{\beta} \qquad \text{eq. (16)}$$

Now, by the extrapolation method

$$Vd = \frac{\text{Dose}}{B} \qquad \text{eq. (18)}$$

Accordingly in terms of the one compartment model a distribution volume may be derived from area measurements and $\beta$ thus:

$$Vd = \frac{\text{Dose}}{\beta.\text{area}} \qquad \text{eq. (19)}$$

For a drug which obeys the two-compartment open model the corresponding relationship is:

$$Vd_{(\text{area})} = \frac{\text{Dose}}{\beta\left\{\frac{A}{\alpha} + \frac{B}{\beta}\right\}} \qquad \text{eq. (20½}$$

Disbribution volume at steady state

An alternative method of expressing distribution volume in a form which takes account of an equilibrium between drug in the blood and drug in the body as a whole is the distribution volume at steady state ($Vd_{ss}$). In the two compartment system this presents a difficulty because as distribution is occurring into the peripheral compartment drug is also lost by elimination from the central compartment. A steady state of equilibrium can only be said to exist when there is no nett transfer of drug between the two compartments. This occurs at only one instant following an intravenous bolus. Nevertheless assuming that nett transfer of drug between the two compartments is zero, $Vd_{ss}$ may be estimated

from the rate constanst as follows: (Riggs, 1963, quoted by Riegelman et al, 1968)

$$Vd_{ss} = \left[\frac{k_{12} + k_{21}}{k_{21}}\right] v_1 \qquad \text{eq. (21)}$$

Although the constraint of no nett transfer of drug between compartments is a theoretical limitation to the use of $Vd_{ss}$ after single intravenous bolus injections, under conditions of constant rate intravenous infusions when equilibrium has been reached, the $Vd_{ss}$ correctly relates the steady state blood concentration to the amounts of drug in the body.

Reigelman et al (1968) pointed out that distribution volumes calculated by the extrapolation and by the area methods are influenced by the elimination processes but it is apparent from eq. 21 that $Vd_{ss}$ is no affected by elimination. Hence factors which alter elimination (other drugs, disease) may influence $Vd_{extrap}$ and $Vd_{area}$ but not $Vd_{ss}$ and in this respect the latter is more acceptable as a constant.

Much of the work to be described is concerned with altering conditions of elimination of drugs which were administered in single doses and all three methods were used to express distribution volume $Vd_{extrap}$, $Vd_{area}$ and $Vd_{ss}$.

### 1.3.3 Clearance

The notion of clearance (CL) was originally developed to describe the elimination of urea by the kidney (Möller, McIntosh and Van Slyke, 1929). Clearance has subsequently been widely adopted in pharmacokinetic work to quantify removal of drug by liver, kidney and other organs and hence to provide an essential parameter for the construction of dosage regimens. In terms of an eliminating organ clearance is defined as the volume of perfusing medium which is effectively

cleared of drug by the organ in unit time; clearance thus has the dimensions of volume and time. Total body clearance is the sum of the clearances of the constituent tissues and organs of the body.

If a drug equilibrates rapidly between blood and tissues after intravenous injection and if after oral administration it is not metabolised before entering the systemic circulation, then its kinetics can often be described by the one-compartment open model with elimination as a first-order process as shown in Figure 1.6. Since the present work is mainly concerned with hepatic eliminatin $k_e$ can here be considered the first-order rate constant for removal of drug by the liver.

In such a system clearance is related to $k_e$ and V, the apparent volume of distribution of the drug as follows:

$$CL = Vk_e \qquad \text{eq. (22)}$$

The relationship of half-life ($t_{\frac{1}{2}}$) to $k_e$ in the model depited in Figure 1.6 is in Figure 1.6 is

$$t_{\frac{1}{2}} = \frac{0.693}{k_e} \qquad \text{eq. (7)}$$

It is thus apparent that in the one compartment model, clearance and half-life are directly related to drug elimination as expressed in eq. (22) and eq. (7) and both parameters thus characterise elimination of drugs which are distributed according to this system.

Many drugs, however, display in the body the distribution characteristics of a two-compartment open system with elimination from the central compartment as shown in Figure 1.8. For drugs which are eliminated only by the liver, $k_e$ in this scheme must also be the first-order rate constant for hepatic elimination.

The general relationship between the dose of drug administered and clearance (Perrier and Gibaldi, 1974) is given by

$$CL = \frac{dose}{AUC_{(o \rightarrow \infty)}} \qquad \text{eq. (23)}$$

where $AUC_{(o \rightarrow \infty)}$ is the area under the blood concentration-time curve from zero time to infinity. Thus for the one-compartment model.

$$AUC = \frac{dose}{V.k_e} \qquad \text{eq. (24)}$$

In the case of the two-compartment system with elimination only from the central compartment ($V_1$)

$$AUC = \frac{dose}{V_1 k_e} \qquad \text{eq. (25)}$$

Hence for this system also

$$CL = V_1 k_e \qquad \text{eq. (26)}$$

indicating that there is a direct relationship between clearance and hepatic drug elimination.

By contrast, however, in multi-compartment systems, half-life is not only a function of the activity of the eliminating organ but it is also influenced by drug distribution. For the two-compartment open system Perrier and Gibaldi (1974) related half-life and the individual rate constants as follows:

$$t_{\frac{1}{2}} = 0.693 \Big/ \left\{ \tfrac{1}{2} \left[ (k_{12} + k_{21} + k_e) - \sqrt{(k_{12} + k_{21} + k_e)^2 - 4\, k_{21}\, k_e} \right] \right\} \qquad \text{eq. (27)}$$

It is clear that in such a complex relationship there would not be a proportionality between change in hepatic elimination and change in half-life. Hence in multi-compartment systems clearance (eq. (26)) can be expected to give a more realistic insight into the elimination characteristics of drugs which are removed by the liver.

1.3.4. Absorption kinetics

The rate at which a drug is absorbed from its point of administration can be determined from the blood concentration-time profile (Nelson, 1961; Loo and Riegelman, 1968). When a drug is taken orally its absorption can usually be described by first-order kinetics, sometimes proceeded by an apparent lag phase. Drug absorption and elimination can thus be depicted as two consecutive apparent first order processes thus:

drug at absorption site $\xrightarrow{k_{ab}}$ drug in body $\xrightarrow{k_d}$ metabolised and excreted drug

If $k_{ab}$ exceeds $k_d$ then the slope of the plot of logarithm of blood concentration against time will eventually be equal to that found after intravenous administration, that is, $-\frac{k_d}{2.303}$. However, if the absorption process is slow and $k_d$ exceeds $k_{ab}$, then the slope of the logarithm of concentration against time plot will be less steep than that obtained after intravenous administration. In the present work, the influence on rate of disappearance from the blood of one drug which is rapidly absorbed from the gut (lignocaine) may be contrasted with one which is slowly absorbed (tocainide).

Determination of the first order absorption rate constant (Notari, 1975; Gibaldi and Perrier, 1975) may be achieved by the method of residuals, by back-extrapolation of the plot of the logarithm of blood concentration versus time to zero time as depicted in Figure 1.9. The differences between the extrapolated line and the observed drug concentration points with respect to time have a slope of $-k_{ab} / 2.303$ from which the absorption rate constant may be calculated.

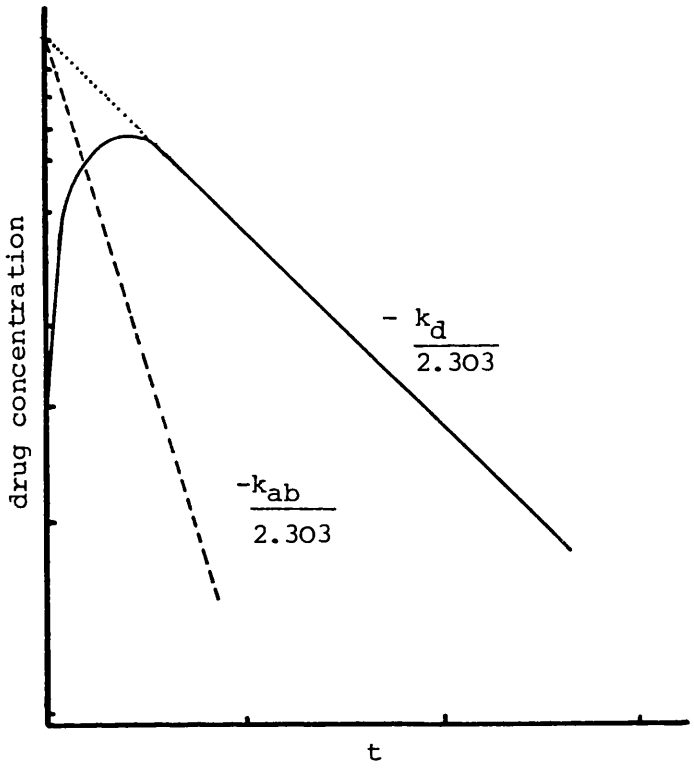



Figure 1.9 Estimation of $k_{ab}$ by the method of residuals (see text)

1.3.5 Systemic availability

Drug availability can be defined as the extent to which a drug, after administration, reaches the point of measurement. The concept of the two-compartment open model commonly proposes that the central compartment comprises the blood and highly perfused tissues including the elminating organs, the kidneys and liver, and it is often assumed that drug is administered into the central compartment. However, it has become clear that the route by which drug reaches the systemic circulation may have an important influence on its kinetics. Drugs such as lignocaine (Boyes, Adams and Duce, 1970); propranolol (Shand, Nicholls and Oates, 1970); propoxyphene (Perrier and Gibaldi, 1972) and nortrypline (von Bahr, Borga, Fellinius and Rowland, 1973), which undergo high hepatic elimination are subject to extensive biotransformation during the initial pass through the liver when they are

administered by the oral route. This phenomenon has been termed the "first-pass" effect. It follows that drugs subject to this effect have a smaller AUC after oral than after intravenous administration of an equivalent dose indicating reduced systemic availability despite complete absorption by the oral route. Thus for such drugs, availability and by implication, pharmacological effect, are influenced by the route of administration (Gibaldi and Perrier, 1974; Shand, Kornhauser and Wilkinson, 1975).

Presystemic elimination raises considerable implications for therapeutics for not only does dosing by the oral route require to be greater than that for intravenous administration to achieve the same effect but the extent of first pass varies between individuals as was shown by Evans and Shand (1973) for propranolol in single and in repetitive dosing. Liver disease which is often associated with reduced first-pass elimination of highly hepatically cleared drugs may further complicate dosing (Wilkinson and Schenker, 1975; Wilkinson and Schenker, 1976).

While the first-pass effect is most often thought of in terms of hepatic elimination, the cells of the gut wall are undoubtedly capable of metabolising certain drugs including isoprenaline (Conway, 1968); chlorpromazine (Curry, D'Mello and Mould, 1971) and salicylamide (George, Blackwell and Davies, 1974). Such drugs also exhibit reduced availability by the oral route despite complete absorption from the gut. In laboratory animals comparison of AUC after drug administration by the oral and intraperitoneal routes can signify significant gut wall metabolism since drug in the peritoneal cavity mainly enters the hepatoportal system but avoids first-pass through gut cells.

1.3.6 Presystemic drug elimination - a compartmental model

A pharmacokinetic approach to the issue of first-pass or presystemic elimination was adopted by Gibaldi, Boyes and Feldman (1971) using a compartmental scheme. These workers proposed the addition to the two-compartment model of a third hepato-portal compartment which is related to the central compartment as shown in Figure 1.10.

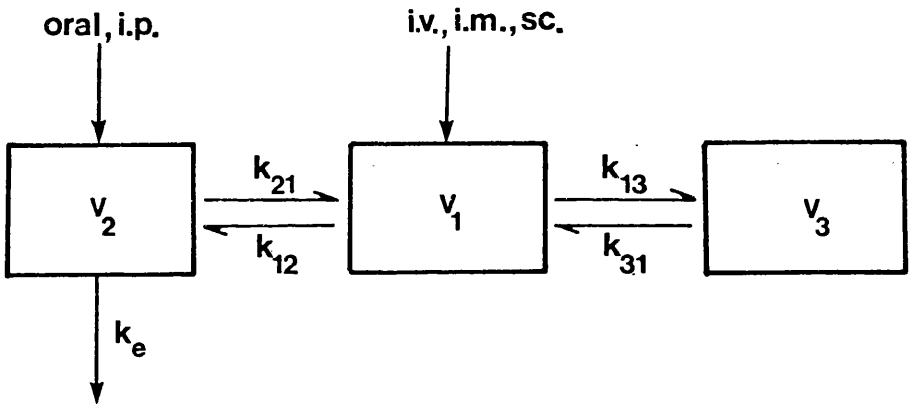



Figure 1.10 First-pass pharmacokinetic model (three-compartment open model)

When drug is administered into compartment 1, equivalent to an intravenous dose, the following relationship was derived:

$$AUC_{i.v.} = D(k_{21} + k_e)/(V_1\ k_{12}\ k_e) \qquad \text{eq. (28)}$$

Where $AUC_{i.v.}$ is the area under the blood concentration-time curve after intravenous administration.

Administration of drug into compartment 2, comparable to the oral route and assuming negligible loss in the gut wall, or to delivery into the hepatic portal vein gives rise to the following relationship:

$$AUC_{p.o.} = D(k_{21})/(V_1\ k_{12}\ k_e) \qquad \text{eq. (29)}$$

where $AUC_{p.o.}$ is the area under the blood concentration-time curve after oral administration.

As previously indicated, the significant feature of drugs which undergo presystemic elimination is that the AUC differs with route of administration. Comparison of AUC by the oral and by the intravenous routes provides a measure of the fraction of drug in the hepatoportal compartment which reaches the central compartment, that is, systemic availability (F) which is derived as follows:

$$F = \frac{AUC_{p.o.}}{AUC_{i.v.}} = \frac{k_{21}}{k_{21} + k_e} \qquad \text{eq. (30)}$$

Multiplication of numerator and denominator of eq. (29) by $V_2$ gives:

$$F = \frac{V_2\ k_{21}}{V_2\ k_{21} + k_e\ V_2} \qquad \text{eq. (31)}$$

If it is assumed that:

a) clearance between compartments is equal in both directions (Perrier and Gibaldi, 1974), i.e. $V_2\ k_{21} = V_1\ k_{12}$ and

b) transfer between compartments 1, and 2 is blood flow limited (Bischoff and Dedrick, 1968) i.e. $V_1\ k_{12} = Q$ where Q is hepatic blood flow, then the following general relationship can be derived from eq.(31).

$$F = \frac{Q}{Q + V_2\ k_e} \qquad \text{eq. (32)}$$

This compartmental model can be used to estimate systemic availability. $V_2\ k_e$ represents clearance of drug from the hepatoportal compartment and since $V_1\ k_{12} = V_2\ k_{21}$, from eq. (29)

$$V_2\ k_e = \frac{D}{AUC_{p.o.}} \qquad \text{eq. (33)}$$

Hence from eq. (32)

$$F = \frac{Q}{Q + (D/AUC_{p.o.})} \qquad \text{eq. (34)}$$

assuming that the dose D is completely absorbed from the gut.

Perrier and Gibaldi (1974) defined $V_2\ k_e$ as the "intrinsic clearance" ($CL_{int}$) of drug from the hepatoportal compartment in that it describes "the inherent ability or capacity of the liver to metabolize a drug assuming the drug is not limited in its access to hepatic cells by distributional factors". It is evident from eq. (33) that with this compartmental model, $CL_{int}$ is dependent only on dose and $AUC_{p.o.}$ and hence

$$AUC_{p.o.} = \frac{D}{CL_{int}} \qquad \text{eq. (35)}$$

Substituting eq. (35) into (eq. (28) permits a definition of availability as

$$F = \frac{Q}{Q + CL_{int}} \qquad \text{eq. (36)}$$

Furthermore from the general formula for clearance eq. (23) and the $AUC_{i.v.}$ given by eq. (28), clearance for this model may be defined as

$$CL = \frac{Q.CL_{int}}{Q + CL_{int}} \qquad \text{eq. (37)}$$

It will be shown that equivalent relationships for availability and clearance may be derived from the "well-stirred" perfusion model (3.1.7).

### 1.3.7 Hepatic drug clearance - perfusion models

The essential value of pharmacokinetic models should lie in their ability to predict changes in drug disposition when the determining conditions change. Compartmental models, while they can be descriptively accurate, are limited in the extent to which they can be manipulated to express changes in drug disposition induced by altered physiological or pathological conditions. Rowland, Benet and Graham (1973) for example have pointed out that the influence of blood flow on steady state clearance cannot be predicted by the compartmental model.

The value of clearance as a parameter by which drug elimination may be expressed has already been referred to and in recent years this approach has been used increasingly by investigators (Rowland et al, 1973; Perrier and Gibaldi, 1974a; Wilkinson and Shand, 1975). In respect of a particular organ, clearance may be defined as that volume of blood from which drug is completely removed in unit time and it is equal to the product of blood flow through the organ (Q) and the extraction ratio (E), hence:

$$CL = QE \qquad \text{eq. (38)}$$

Clearance is a parameter of the efficiency with which drug is removed from the blood irrespective of the extent to which drug distributes to other tissues and is thus independent of whichever model may be selected to describe its pharmacokinetics. In the instance in which a drug is eliminated exclusively by a single organ, the characteristics of drug elimination by the body as a whole are expressed by the clearance of drug by that organ. Thus for a drug which is eliminated solely by the liver, body clearance is equivalent to hepatic clearance.

The fundamental biological determinants of drug elimination by the liver are:

a) hepatic blood flow;

b) the inherent ability of the liver to remove drug from the blood (hepatic metabolic and transport processes).

c) drug binding to plasma proteins and cellular constitutents of the blood.

The "well stirred" model

With the objective of incorporating such variables into a predictive scheme, Rowland (1972a) and Rowland et al (1973) proposed a perfusion limited model which is depicted in Figure 1.11.

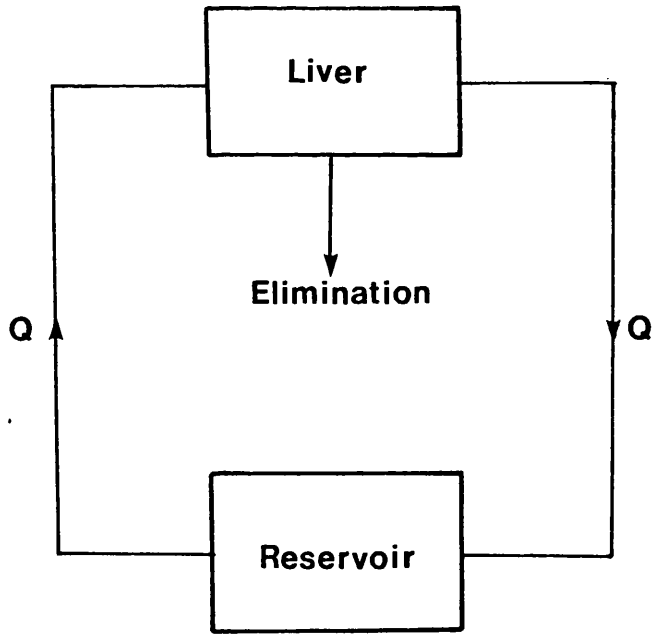



Figure 1.11 Perfusion limited model

In this model, blood perfuses an eliminating organ, which in the present context may be taken to represent the liver, and is then returned to a reservoir which represents the rest of the body. Q is the blood flow entering and leaving the organ. It is assumed that:

1. that complete mixing takes place between the hepatic arterial and the hepatic portal supplies before blood enters the hepatic sinuoids. There is evidence that this is so (Greenway and Stark, 1971).
2. that the liver is a single well-stirred compartment.
3. that blood entering and leaving the liver contains drug in the unbound state and drug bound to plasma proteins and blood cells.
4. that drug distribution in the liver is rapid, such that drug in the emergent venous blood is in equilibrium with that in the liver.
5. that only drug in the unbound state can cross cell membranes.
6. that there is no barrier to diffusion between drug in the blood and the enzymes within the liver cells i.e. that distribution is limited only by perfusion.
7. that the drug elimination rate is a function of the unbound drug concentration in the fluid surrounding the enzymes.
8. that the concentration of drug in the blood entering the liver is equal to that in the reservoir.
9. that the concentration of drug in the blood leaving the liver is equal to that entering the reservoir.

Various relationships may be derived on the basis of this model. Consider drug at a steady state concentration in the blood entering the liver; the quantity of drug which is eliminated from the blood in unit time is given by:

$$CL_h C_s = \frac{V_{max}\ f_B\ C_{hv}}{K_m + f_B\ C_{hv}} \qquad \text{eq. (39)}$$

where: $CL_h$ is hepatic drug clearance from the blood.

$C_s$ is the concentration of drug in the blood entering the liver (systemic)

$C_{hv}$ is the concentration of drug in the emergent venous blood (hepatic vein)

$f_B$ is the unbound fraction of drug in the blood

$V_{max}$ and $K_m$ are the Michaelis constants for the removal process.

In most circumstances the concentration of unbound drug in the liver ($f_B\ C_{hv}$) is much less than $K_m$ and since drug elimination is assumed to be a first order process eq. (39) reduces to

$$CL_h\ C_s = \frac{V_{max} \cdot f_B\ C_{hv}}{K_m} \qquad \text{eq. (40)}$$

The constants $V_{max}$ and $K_m$ may be combined into a single clearance term which is a measure of intrinsic hepatocellular enzyme activity independent of blood flow and binding. This is the "intrinsic free drug clearance" $CL_{int(free)}$ and it relates the rate of hepatic elimination to the concentration of drug surrounding the hepatic enzymes. It is thus defined as the volume of liver water which is effectively cleared of drug in unit time (Shand et al, 1975). Eq. (40) may then be rearranged:

$$CL_h = f_B \cdot CL_{int(free)} \cdot \frac{(C_{hv})}{C_s} \qquad \text{eq. (41)}$$

Data relating organ clearance of drug to organ blood flow often involve measurements in whole blood, whereas intrinsic clearance as defined above refers only to unbound drug. It was therefore proposed by Kornhauser, Wood, Vestal, Wilkinson, Branch and Shand (1978) that intrinsic clearance calculated on the basis of free drug be called intrinsic free drug clearance $CL_{int(free)}$ and that intrinsic clearance on the basis of total drug concentration be termed total

intrinsic clearance $CL_{int(tot)}$. The relationship between $C_{int(free)}$ and $CL_{int(tot)}$ is

$$CL_{int(tot)} = CL_{int(free)} \cdot f_B \qquad \text{eq. (42)}$$

The term $\frac{C_{hv}}{C_s}$ represents that fraction of drug in the blood which is not removed by the liver i.e. which becomes available to the systemic circulation, that is F. By definition, the value F must equal 1-E where E is the hepatic extraction of the drug. Thus by substitution in eq. (38) and (41)

$$QE = f_B \, CL_{int(free)} \cdot (1-E) \qquad \text{eq. (43)}$$

hence

$$E = \frac{f_B \, CL_{int(free)}}{Q + f_B \, CL_{int(free)}} \qquad \text{eq. (44)}$$

and

$$CL_h = QE = Q\left[\frac{f_B \, CL_{int(free)}}{Q + f_B \, CL_{int(free)}}\right] \qquad \text{eq. (45)}$$

It is apparent that this perfusion model permits an analysis of clearance of drug from the blood in a relationship which includes three of the fundamental biological variables of hepatic drug elimination namely

a) blood flow;

b) the intrinsic ability of the liver to remove drug from the blood and

c) binding of drug within the blood.

The general equation (45) allows an assessment of the influences of blood flow, drug metabolism and drug binding on AUC for different modes of administration.

1. intravenous bolus dose

Estimation of $CL_h$ across the liver according to eq. (38) presents technical difficulties *in vivo* but when a drug is cleared exclusively by the liver, the total body clearance ($CL_t$) may be determined from an intravenous dose according to the general relationship eq. (23), thus:

$$CL_t = \frac{D_{i.v.}}{AUC_{i.v.}} \qquad \text{eq. (46)}$$

where $D_{i.v.}$ is the intravenous dose and $AUC_{i.v.}$ is the area under the blood concentration time curve after intravenous administration, extrapolated to infinity.

Then substituting eq (45) into eq (46) and rearranging

$$AUC_{i.v.} = \frac{D_{i.v.}}{Q\left[\frac{CL_{int(free)} \cdot f_B}{Q + CL_{int(free)} \cdot f_B}\right]} \qquad \text{eq. (47)}$$

In the case of a highly cleared drug, when $Q << f_B\ CL_{int(free)}$ eq. (47) may be reduced to:

$$AUC_{i.v.} = \frac{D_{i.v.}}{Q} \qquad \text{eq. (48)}$$

Thus for this category of drug AUC is influenced by alteration in liver blood flow but effects of alteration in hepatic metabolism on AUC are minimal.

By contrast for a drug which is poorly cleared, that is when $Q >> f_B\ CL_{int(free)}$ eq. (47) may be reduced to

$$AUC_{i.v.} = \frac{D_{i.v.}}{f_B\ CL_{int(free)}} \qquad \text{eq. (49)}$$

Hence for this category of drug, AUC is influenced only by changes in the intrinsic metabolic clearance of the drug.

2. constant intravenous infusion

At steady state after a consistant rate intravenous infusion ($R^o$)

$$C_{ss} = \frac{R^o}{CL_t} = \frac{R^o}{Q\left[\frac{CL_{int(free)} \cdot f_B}{Q + CL_{int(free)} \cdot f_B}\right]} \qquad \text{eq. (50)}$$

Where $C_{ss}$ is the total blood concentration at steady state.

Since eq. (50) is similar to that for AUC after an intravenous bolus it is apparent that the same conclusions may be drawn.

3. single oral dose

When a drug is absorbed from the gut it is carried in the portal system to the liver where a fraction of the dose is eliminated (E) and the remainder (1-E) becomes available to the systemic circulation. For a drug which is removed totally by hepatic metabolism the fraction 1-E is then cleared from the blood at a rate equal to total systemic clearance eq. (23). Consequently:

$$AUC_{p.o.} = \frac{D_{p.o.} \cdot (1-E)}{QE} \qquad \text{eq. (51)}$$

where: $AUC_{p.o.}$ is the area under the blood concentration-time curve after oral administration, extrapolated to infinity and

$D_{p.o.}$ is the oral dose

Hence clearance by the oral route is given by:

$$CL_{p.o.} = \frac{D_{p.o.}}{AUC_{p.o.}} = \frac{QE}{1-E} \qquad \text{eq. (52)}$$

Substituting for E in the general relationship eq. (45) thus gives:

$$CL_{p.o.} = CL_{int(free)} \cdot f_B \qquad \text{eq. (53)}$$

thus

$$AUC_{p.o.} = \frac{D_{p.o.}}{CL_{int(free)} \cdot f_B} \qquad \text{eq. (54)}$$

4. repeated oral dosing

If an oral dose of a drug is given repeatedly at intervals of time T, the mean blood concentration at steady state ($C_{\overline{ss}}$) is given by:

$$C_{\overline{ss}} = \frac{D_{p.o.}\ (1-E)}{CL_t \cdot T} \qquad \text{eq. (55)}$$

where T is the dosing interval.

Assuming all the drug is absorbed from the gut, that there is no loss of drug prior to reaching the liver and that the liver alone is responsible for drug elimination i.e., that $CL_t = QE$

$$C_{\overline{ss}} = \frac{D_{p.o.}\ (1-E)}{QE \cdot T} \qquad \text{eq. (56)}$$

Rearrangement of the general relationship in eq. (43) yields

$$f_B\ CL_{int(free)} = \frac{QE}{1-E} \qquad \text{eq. (57)}$$

Substitution of eq (56) into (57) gives

$$C_{\overline{ss}} = \frac{D_{p.o.}}{f_B\ CL_{int(free)} \cdot T} \qquad \text{eq. (58)}$$

Hence for both highly cleared and poorly cleared drugs, the steady state blood concentration is dependent on intrinsic clearance and is independent of flow.

The "well stirred" model has been considered in some detail here because it has been most widely examined. There is, however, another less frequently studied model which also relates blood flow, drug binding and metabolism to hepatic drug extraction, namely the "parallel tube" model.

The "parallel tube" model

The "parallel tube" model was proposed by Winkler, Keiding and Tygstrup (1973) and Winkler, Bass, Keiding and Tygstrup (1974). The assumptions and predictions of this model have been examined in detail by Pang and Rowland (1977a). The "parallel tube" model describes

the sinusoids of the liver as a series of identical and parallel cyclinders in which the enzymes are distributed evenly in the hepatic cells surrounding the cylinders. Blood flows along the cylinders only in one direction and at any point in the cylinder, drug in the blood is in equilibrium with that at the site of enzyme action. Hence the concentration of unbound drug declines within the cylinder along the direction of flow as depicted in Figure 1.12

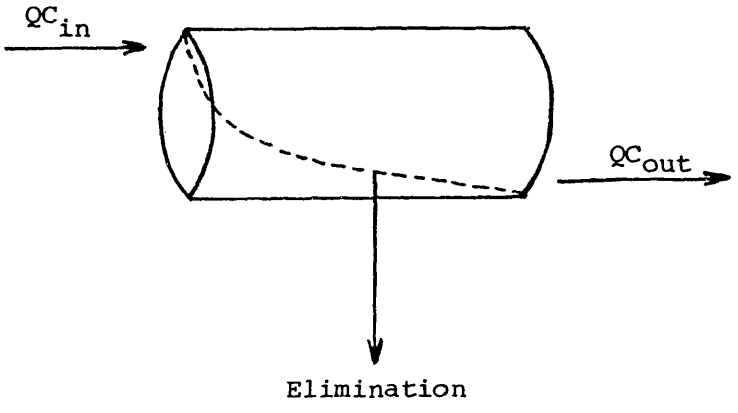



Figure 1.12 Diagrammatic represenation of hepatic elimination in the "parallel tube" model

It is assumed that the total metabolising ability of the liver is the sum of the metabolising capacity of individual cylinders. The parallel tube model shares a number of assumptions with the well-stirred model namely:-

1. that complete mixing of blood takes place between the hepatic arterial and the hepatic portal supplies before blood enters the sinusoids;
2. that blood entering and leaving the liver contains drug in the unbound state and drug bound to plasma proteins and blood cells;
3. that there is no barrier to diffusion between drug in the blood and the enzymes within the liver cells, i.e. that distribution is limited only by perfusion;
4. that only drug in the unbound state can cross cell membranes, i.e. that the drug elimination rate is a function of the unbound drug concentration in the fluid surrounding the enzymes.

The assumptions in the parallel tube model of progressive decline of unbound drug concentration with blood flow along each cyclinder is in distinct contrast with the well-stirred model concept of the liver as a homogeneous entity in which unbound drug concentration is equal throughout. Accordingly differences may be anticipated in the predictions made by each model when the major physiological determinants alter.

In a theoretical analysis of the well stirred model (Model I) and the parallel tube model (Model II), Pang and Rowland (1977a) derived relationships for pharmacokinetic parameters (hepatic extraction and clearance, availability, AUC after single doses and steady state blood concentration after both intravenous and oral dosing), for both models in terms of blood flow, intrinsic clearance and drug binding, and compared the predictions of the models when these determinants were altered. (Table 1.2). For change in blood flow no marked difference was found in the predictions of these two models in respect of extraction ratio, clearance, AUC following a single intravenous dose and steady state drug concentation following a constant intravenous infusion.

However, a significant difference between the models lies in their predictions for AUC following a single oral dose of a highly cleared drug. In terms of Model I $AUC_{p.o.}$ is dependent only on dose and intrinsic clearance and no change in area occurs with alteration in blood flow whereas by Model II, $AUC_{p.o.}$ is affected by change in blood flow. Most available experimental data have been related to the predictions of Model I. Using the isolated perfused rat liver preparation, Branch et al (1973) showed that propranolol clearance was dependent on hepatic blood flow as predicted by Model I but this evidence does not favour one or other model since hepatic clearance is a poor discriminator between the models. However, using the same preparation Shand et al (1975) provided evidence that the steady state blood concentration of lignocaine after oral administration was independent of flow compatible with the predictions of Model I. In a direct comparison of the predications of both models using the perfused rat liver in situ preparation Pang and Rowland (1977b) found that their data with lignocaine supported the assumption of Model I rather than Model II. In contrast Bennett, Bending, Steiner and Rowland (1978) studying lignocaine in man when hepatic blood flow was altered by change of posture, found that AUC after oral administration did change with alteration in blood flow as predicted by Model II.

The present work which examines the influence of alteration in blood flow and metabolism on drug kinetics offers a further opportunity to compare the predictions of these models.

Table 1.2 Interrelationships of hepatic blood flow (Q), ratio of drug concentration unbound in plasma to that in whole blood ($f_B^*$), maximum intrinsic clearance ($CL_{int}$), and various pharmacokinetic parameters under linear kinetic conditions according to Models I and II

| Parameter | Symbol | Basic equation | Interrelationships defined by | |
|---|---|---|---|---|
| | | | Model I | Model II |
| Extraction ratio | $E$ | | $\frac{f_B CL_{int,l}}{f_B CL_{int,l} + Q}$ | $1 - e^{-(f_B CL_{int,l}/Q)}$ |
| Hepatic clearance | CL | $QE$ | $Q\frac{f_B CL_{int,l}}{f_B CL_{int,l} + Q}$ | $Q[1 - e^{-(f_B CL_{int,l}/Q)}]$ |
| Total area under blood drug concentration–time curve following a single intravenous dose | $AUC_{i.v.}$ | $\frac{dose}{CL}$ | $\frac{dose(f_B CL_{int,l} + Q)}{f_B CL_{int,l} Q}$ | $\frac{dose}{Q[1 - e^{-(f_B CL_{int,l}/Q)}]}$ |
| Steady-state blood drug concentration following constant intravenous infusion | $C_{B,ss,inf}$ | $\frac{R_{inf}}{CL}$ | $\frac{R_{inf}(f_B CL_{int,l} + Q)}{f_B CL_{int,l} Q}$ | $\frac{R_{inf}}{Q[1 - e^{-(f_B CL_{int,l}/Q)}]}$ |
| Availability | $F$ | $1 - E$ | $\frac{Q}{f_B CL_{int,l} + Q}$ | $e^{-(f_B CL_{int,l}/Q)}$ |
| Steady-state output drug concentration from liver (hepatic venous blood) | $C_{Out}$ | $C_{In}(1 - E)$ | $\frac{C_{In} Q}{f_B CL_{int,l} + Q}$ | $C_{In}\, e^{-(f_B CL_{int,l}/Q)}$ |
| Total area under blood drug concentration–time curve following a single oral dose | $AUC_{oral}$ | $\frac{F \cdot dose}{CL}$ | $\frac{dose}{f_B CL_{int,l}}$ | $\frac{dose[e^{-(f_B CL_{int,l}/Q)}]}{Q(1 - e^{-(f_B CL_{int,l}/Q)})}$ |
| Steady-state blood drug concentration following constant oral administration | $\bar{C}_{B,ss,oral}$ | $\frac{F \cdot dose/\tau}{CL}$ | $\frac{dose/\tau}{f_B CL_{int,l}}$ | $\frac{[dose/\tau][e^{-(f_B CL_{int,l}/Q)}]}{Q[1 - e^{-(f_B CL_{int,l}/Q)}]}$ |

* Pang and Rowland (1977a)

$CL_{int,1}$ intrinsic hepatic drug clearance when operating under linear conditions

$R_{inf}$ constant intravenous infusion rate

$\tau$ interval between doses

$C_{in}$ $C_{out}$ concentration of drug entering and leaving the liver, respectively

Blood binding and hepatic extraction

The relationship between drug binding and hepatic extraction with perfusion models has been discussed in detail by (Wilkinson and Shand (1975) and by Pang and Rowland (1977). In respect of elimination of unbound drug two types of hepatic extraction may be considered:

a) restrictive in which elimination is limited to the circulating free drug and hence $E<f_B$

and

b) non-restrictive in which extraction is sufficiently avid to remove bound as well as unbound drug, hence $E>f_B$.

The present study is concerned with one drug in which extraction would be expected to be restrictive (tocainide, $f_B \simeq 0.5$, $E \simeq 0.06$) and one in which extraction would be expected to be non-restrictive (lignocaine, $f_B \simeq 0.7$, $E \simeq 0.98$). In the present work drug binding to cells or plasma proteins was not measured and the implication of these observations are considered in the section on Discussion (Chapter VI).

Blood versus plasma measurements

Since CL is an index of the efficiency of drug removal from blood by the eliminating organ, e.g. liver rather than from plasma or liver water, in this work measurements of total drug in blood were used (Rowland, 1972b).

### 1.3.8 Hepatic blood flow

Measurement of liver blood flow

Many techniques have been used to estimate liver blood flow; the number of methods which has been developed reflect the fact that no single technique is widely accepted as satisfactory. Ideally the measurement of liver blood flow should be non-invasive and the estimation should be carried out under normal physiological conditions. Techniques for measuring liver blood flow have recently been reviewed (Ohnhaus, 1979) and include:

a) the electromagnetic flowmeter

This method is based on the induction of an electrical potential by a conducting substance moving at right angles to the lines of force of a magnetic field. The conductor is the stream of blood passing between the poles of a magnet and the induced current is led off by electrodes placed across the stream; the current generated is proportional to the velocity of the stream. Hepatic artery and portal flow can be estimated separately but it is an invasive method.

b) the heat exchange method

A thermocouple heated by a constant current is implanted into the liver and is cooled by the flow of blood; change in blood flow is reflected in change in the current. The method is only semiquantitative and only change in liver blood blow can be measured but it can be used in long term experiments in animals (Ohnhaus, Emons and Breckenridge, 1970).

c) inert gas technique

Inert gases such as $^{85}Kr$ (Tobias, Jones, Lawrence and Hamilton, 1949) or $^{133}Xe$ (Conn, 1955) can be given by injection into the hepatic artery or portal vein or by inhalation from a spirometer in a closed system. A recording is made of the wash-out over the liver by external counting using a scintillation detector or gamma camera. The inhalation technique is non-invasive and is applicable several times to the same individual.

d) labelled colloid uptake

A rapid single injection of a radioactively-labelled colloid is given e.g. $^{32}P$ labelled chromic phosphate, $^{198}AU$ or human serum albumin labelled with $^{131}I$ (Dobson and Jones, 1952; Caesar, Shaldon, Chiandussi, Guevara and Sherlock, 1961). These substances are taken up by the Kupffer-cellsof the liver and the disappearance rate, which can be obtained from blood sampling from a peripheral vein or by external

counting using a gamma counter or scintillation collimeter, is used to estimate hepatic blood flow. The necessity of using radio-active substances is a disadvantage when the technique is used in man.

e) dye clearance methods

An indicator dye is infused continuously into a peripheral vein and liver blood flow can be measured from the formula:

$$Q = \frac{e}{C_a - C_{hv}}$$

where Q is hepatic blood flow, e the elimination rate of the indicator, $C_a$ the hepatic areterial concentration and $C_{hv}$ the hepatic venous concentration. At equilibrium and assuming no extrahepatic elimination e is equal to the infusion rate (I), then

$$Q = \frac{I}{c_a - C_{hv}}$$

Bromsulfphthalein, rose bengal and indocyanine green which are dyes eliminated exclusively by the liver have been used (Simpson, Erzow and Sapirstein, 1954; Ketterer, Weigland and Rappaport, 1960). The method is invasive requiring catheterisation of a peripheral artery and the hepatic vein.

Methods used for calculation of liver blood flow in present study

f) lignocaine clearance

If a drug is completely absorbed from the gut and is cleared only by the liver, then total body clearance equals hepatic clearance. Thus from the general relationships in eq. (23) and eq. (38)

$$CL_t = CL_h = \frac{Dose_{i.v.}}{AUC_{i.v.}} = QE$$

$$\text{and } F = \frac{AUC_{p.o.}}{AUC_{i.v.}}$$

but substitution and rearrangement

$$Q = \frac{\text{Dose}}{AUC_{i.v.} - AUC_{p.o.}} \qquad \text{eq. (60)}$$

Lignocaine satisfies the above criteria and has been found after intravenous and oral administration to man to give values comparable to those obtained with other techniques (Bennett et al, in press; Perucca and Richens, 1979). In the present work this method has been used to estimate liver blood flow in the intact rat, an approach which has not previously been used in this animal.

g) radioactively labelled microspheres

With this method; i) the cardiac output;

ii) the distribution of regional blood flow

and iii) the fractional shunting of blood through arteriovenous anastomoses can be determined.

The origin of the method was the intravenous injection of radioactive substances which are homogenously distributed in the body and taken up by the organs during its first transit. Organ blood flow is determined from the distribution of these substances within the organs. Earlier work by Sapirstein (1956) used radioactive meterial such as $^{42}$KCl, $^{86}$Rb Cl or $^{131}$I-antipyrene. However, after radioactive microspheres labelled with different isotopes such as $^{51}$Cr, $^{141}$Ce, $^{85}$Sr and $^{125}$I were introduced by Rudolf and Heymann (1967) as the indicator substances, many experiments have been performed in small laboratory animals using this method (Neutze, Wyler and Rudolph, 1968; Mendell and Hollenberg, 1971; Sasaki and Wagner, 1971; Bartrum, Berkowitz and Hollenberg, 1974 and McDevitt and Nies, 1976; Yates, Hiley and Back, 1979). The microspheres are of such a size (9-50 μ) that after injection they are distributed according to blood flow but are trapped in capillaries of the perfused tissue. The number of particles entering each organ during the first transit is proportional to the blood flow

to that organ. The distribution of radioactivity in the excised tissues is determined using a radiation detector such as a gamma-counter. Cardiac output and regional blood flow can then be determined (see Chapter 2.11). This method is based on the assumptions (Wagner, Rhodes, Sasaki and Ryan, 1969) that: i) microspheres must be well mixed with blood; therefore they are usually injected through a catheter placed in the left ventricle;

ii) the circulation should not be disturbed by the administration of microspheres and;

iii) the microspheres should all be trapped in the capillaries during the first circulation.

If the blood flowing through the liver is completely cleared of the radioactive micorspheres during a single passage, the equation for conservation of mass under the assumptions of steady flow and complete mixing of indicator is (Archie, Fixler, Ullyot, Hoffman, Utley and Carlson, 1973)

$$\text{flow} = I/\int_0^\infty C(t)dt$$

where I is the quantity of spheres, C(t) is the concentration of indicator at any time, and t is time. Microspheres are injected directly to the heart, an arterial sample is collected at a known constant rate ($Q_{ar}$) and the sample radioactivity ($I_{ar}$) is counted. Then, if the amount of radioactivity injected ($I_{tot}$) and the radioactivity in the liver ($I_{hep}$) are known, the cardiac output (CO) and liver blood flow (Q) are determined as follows:

$$Co = I_{tot}\ (Q_{ar}/I_{ar})$$

and $$Q = I_{hep}\ (Q_{ar}/I_{ar}).$$

# CHAPTER II

## Materials and Methods

## 2.1 Compounds

Lignocaine hydrochloride [ 2-diethylamino-N-(2,6-dimethylphenyl) acetamide hydrochloride ], tocainide hydrochloride [ 2-amino-N-(2,6-dimethylphenyl) propanamide hydrochloride ], 2-methylethylamino-N-(2,6-dimethylphenyl) acetamide hydrochloride, W36149 hydrochloride [ 2-amino-N(2,6-diemthylphenyl) butanamide hydrochloride ] were generous gifts from Astra Chemicals Ltd., Watford, sotalol hydrochloride was supplied by the Bristol-Myers Company Ltd., Windsor, and polystyrene microspheres, 14.8 ± 1.1 μm diameter ($^{85}Sr$ labelled) were purchased from 3M Co., St. Paul, MN, U.S.A.

In addition, phenobarbitone sodium (The British Drug Houses Ltd., Poole), benzo (α) pyrene (3,4-benzpyrene, Sigma Chemical Company, London), pentobarbitone sodium (Sagatal, May and Baker, Dagenham) and corn oil (Mazola brand, Best Foods) were used.

Chemicals and reagents used throughout, whenever possible, were of Analar grade. They were obtained from commercial sources and purified before use where appropiate.

## 2.2 Animals

Male albino rats of the CFY strain (Anglia Laboratory Animals, Huntingdon) weighing between 350-500 g were used throughout. Rats were housed in controlled photoperiod conditions (14 hour light, 10 hour dark) at a temperature of 21°C with food (Oxoid diet 41B) and water available ad libitum until the experimental conditions below were imposed.

## 2.3 Dosage and administration of compounds

For all experiments dosing was on a weight related basis and the stated weights of lignocaine and tocainide refer to the base form,

although the drugs were administered as the hydrochloride. Drugs were dissolved in physiological saline for intravenous and intraperitoneal injection and were dissolved in distilled water for oral administration. 3,4-Benzpyrene was dissolved in corn oil by heating at 50°C for 1 hour.

Single dose studies

Animals dosed orally were given either lignocaine (50-90 $mg.kg^{-1}$) or tocainide (50-400 $mg.kg^{-1}$) by direct oesophageal intubation of the manually-restrained animal, using an adapted, blunt, stainless steel needle fitted to the appropriate size of syringe. These rats were starved overnight (approximately 20 hours) prior to drug administration and the fast was continued for a further 4 hours after which they were allowed access to food. Free access to water was permitted at all times. Rats which received lignocaine (2.5-10.0 $mg.kg^{-1}$) or tocainide by the i.v. route (5.0-50.0 $mg.kg^{-1}$) received a bolus injection via a tail vein, the duration of the injection being one minute. These animals were allowed free access to food and water throughout the experiments.

Multiple dose studies

For the multiple i.v. dosing experiments, rats were given tocainide (20 $mg.kg^{-1}$) every 8 hours for seven doses and free access to food and water was permitted throughout. When tocainide was given in multiple oral doses (200 $mg.kg^{-1}$, every 12 hours) the rats were fasted for 16 hours before the first dose of tocainide was given and for the first 4 hours after each subsequent dose, following which 5 g of food was given to each animal and the excess if any was taken away after 2 hours so allowing a fast of 6 hours before the next dose.

Enzyme induction and liver blood flow studies

Phenobarbitone was dissolved in isotonic saline to give a solution of 100 $mg.ml^{-1}$ and rats received 100 $mg.kg^{-1}$ daily for 4 days by intraperitoneal injection. 3,4-benzpyrene was dissolved in corn oil to give a solution containing 8 $mg \cdot ml^{-1}$ and rats received a dose of 80 $mg.kg^{-1}$ by a single intraperitoneal injection. Sotalol hydrochloride was dissolved in isotonic saline to give a solution containing 100 $mg.ml^{-1}$ and rats received doses of 100 $mg.kg^{-1}$ by intraperitoneal as described in Chapter 5. Two control groups were used: one group received isotonic saline (1 $ml.kg^{-1}$ daily for 4 days) and the other group received corn oil (10 $ml.kg^{-1}$) in both instances by intraperitoneal injection. Rats were then allocated to pharmacokinetics studies of lignocaine and tocainide, blood flow studies, biochemical investigation or determination of pentobarbitone sleeping time.

## 2.4 Collection of rat urine

Following dosing, animals were housed singly in metabolism cages (7.5" diameter) fitted with a 0.5" mesh floor, placed over a 8" diameter polyethylene funnel designed so that urine could be collected separately from faeces. Urine was collected at 0-6, 6-12 and 12-24 hr after dosing. At the end of each collection period the funnel was throughly rinsed with distilled water and the washings were added to the collected urine.

## 2.5 Collection of blood samples

Throughout the studies 0.3-0.5 ml samples of whole blood were taken from the tip of the tail of each animal at specified times. Animals were placed in all metal restraining cages that allowed minimal movement and free access to the tail. The tip of the tail was removed with a scalpel blade and blood was collected into polyethylene tubes containing approximately 10 µl of heparin (5000 units $ml^{-1}$). Drainage of blood into the collecting vessel was aided by gentle stroking of the tail with the fingers in a base-to-tip direction. Blood was collected during a period of 45 to 60 seconds for each sample.

## 2.6 Storage of biological samples

Blood samples were stored in heparinized polyethylene tubes (Sarstedt, Leicester) at -20°C. An aliquot of the measured volume of urine (+ washings) and homogenized tissues were stored in 6 ml. polyethylene pill tubes (Ventura Plastic Ltd., Chessington) at -20°C without pH adjustment prior to analysis.

## 2.7 GLC analysis

### 2.7.1 Gas-liquid chromatography

A PYE Unicam 104 chromatograph equipped with a flame ionization detector was used. The chromatographic column was of glass, 4 mm in inner diameter and 1.6 m in length.

Lignocaine

Column packing: 5% KOH + 0.75% Carbowax 20 M coated on Chromosorb G AW-DMCS (85-100 mesh)

Operating conditions: Oven temperature = 180°C

Injector port = 210°C

Tocainide

Column packing: 3% OV-17 coated on Chromosorb W-HP (80-100 mesh)

Operating conditions: Oven temperature = 195°C

Injector port = 275°C

Conditioning of columns

The packed columns were conditioned prior to use as follows: after initial heating at 50°C for 60 min the oven temperature was raised by 1°C $min^{-1}$ to 100°C and maintained at that temperature for 60 min, then increased by 1°C $min^{-1}$ to 200°C (lignocaine) or 250°C (tocainide) for 24 hr. Throughout conditioning the columns were perfused with argon at a flow rate of 15 ml.$min^{-1}$. The detector temperature was that of the oven. The columns were silanized *in situ* with Silyl-8 (Pierce). Hydrogen and air were adjusted to give optimum recorder response and in these analyses the flow rate was for $H_2$, 50 ml $min^{-1}$ and for air, 400 ml $min^{-1}$. Argon was used as the carrier gas at a flow rate of 40 ml $min^{-1}$.

Under these conditions the retention times were 6 min 10 sec (lignocaine), 4 min 30 sec [2-methylethyl-amino-N-(2,6-dimethylphenyl) acetamide], 3 min 30 sec (tocainide) and 4 min 15 sec (W 36149).

2.7.2. Estimation of lignocaine by gas-liquid chromatography

Lignocaine in blood and urine was assayed by GLC according to the method of Mather and Tucker (1974).

0.05 to 0.5 ml of biological material (whole blood or urine) in a 15 ml stoppered round-bottomed glass tubes was mixed with 2-methyl-ethyl-amino-N-(2,6-dimethylphenyl) acetamide HCl as the internal standard (1 µg, 50 µl of a 20 µg/ml solution), made alkaline with 1 M NaOH (100 µl), extracted with ether (3 ml) by stirring on a vortex

mixer for 30 sec and then centrifuged. The upper organic phase was transferred in a pasteur pipette to a 10 ml glass stoppered centrifuge tube containing 1 M hydrochloric acid (200 µl) and the phases were mixed and separated as before.

The organic phase was discarded and the aqueous phase was heated to about 60°C by placing the test-tube in a water bath for several minutes in order to allow residual ether to evaporate. After it had cooled, 2 M NaOH (200 µl) was added and lignocaine was extracted with redistilled methylene chloride (25 µl). The phases were separated by centrifugation. Aliquots (1-2 µl) of the organic phase were withdrawn with a microsyringe and taken for GLC analysis.

Lignocaine concentrations in biological fluids were measured using a previously established calibration curve constructed by analyses of known amounts of lignocaine added to normal blood or urine and plotting the peak height ratio (lignocaine : internal standard) versus the lignocaine concentration (see Figure 2.1). The standard curve was linear over a range of 0.05 to 8.00 µg of lignocaine $ml^{-1}$. The coefficients of variation of the assay were as follows: 0.05 µg $ml^{-1}$ (8.29%), 0.50 µg $ml^{-1}$ (3.59%), 0.75 µg $ml^{-1}$ (3.22%), 1.00 µg $ml^{-1}$ (2.12%) and 2.00 µg.$ml^{-1}$ (2.89%) with n = 5 for each concentration.

During determination of lignocaine in biological samples in no case was a peak observed at the retention time of lignocaine or of the internal standard in blank samples and identical linear calibration curves were acheived whether lignocaine was placed in blood, in plasma or in distilled water.

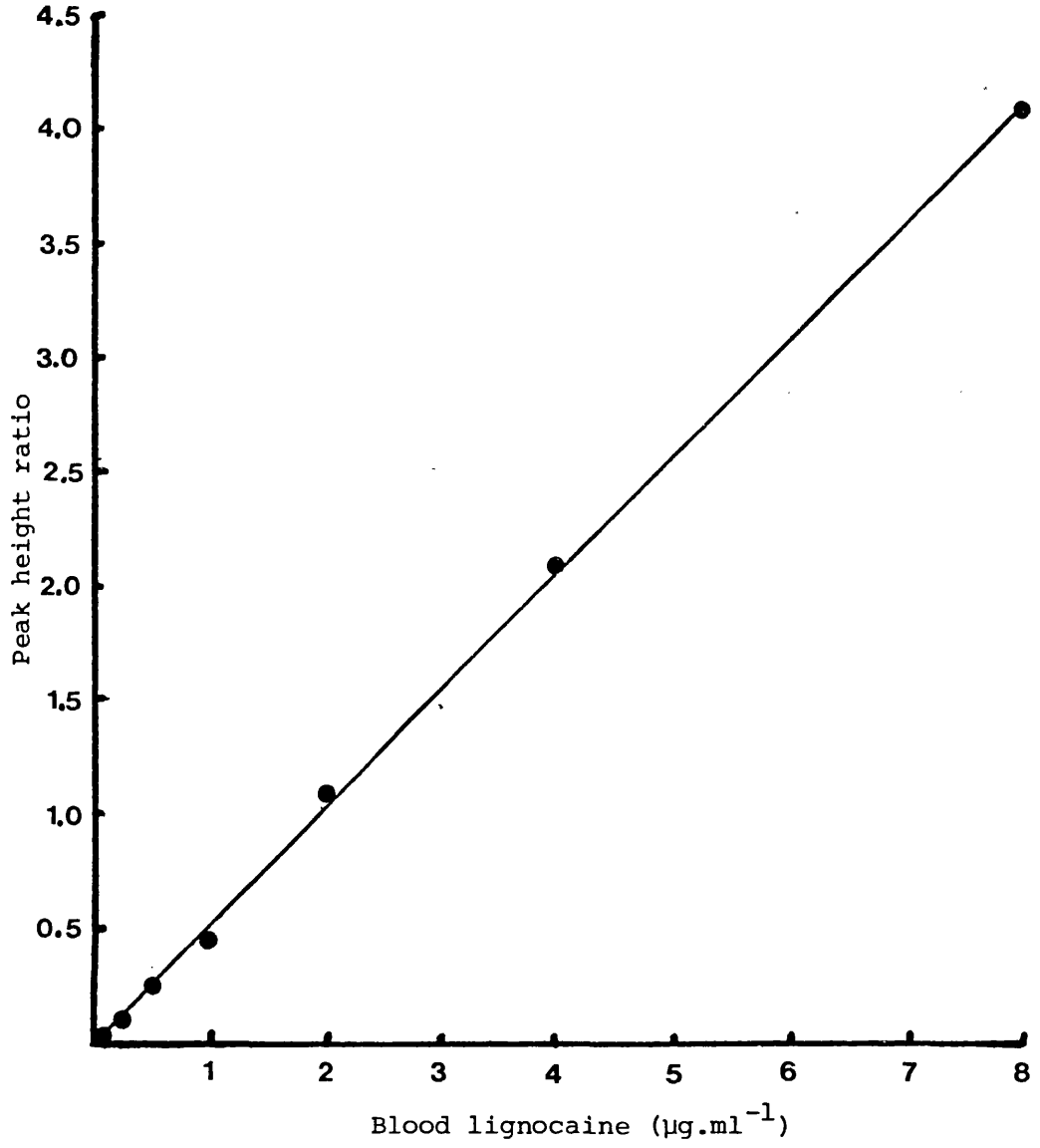



Figure 2.1 Standard calibration curve for lignocaine in whole blood

2.7.3 Estimation of tocainide by gas-liquid chromatography

Whole blood or urine (0.05-0.5 ml) was placed in a 15 ml stoppered round bottomed-glass tube, and an aqueous solution of 5 µg W36149 as the internal standard, (50 µl of a 100 $\mu g.ml^{-1}$ solution) and 1 ml of 1 M NaOH were added, together with distilled water to make a total volume of 5 ml. The tube was vortex-mixed for 10 sec and methylene chloride (5 ml) was added. The tube was capped with a teflon stopper, shaken mechanically for 10 min, and centrifuged for 20 min. The aqueous layer was removed by aspiration and the organic phase was transferred to a clean 10 ml glass stoppered centrifuge tube. The organic phase was then evaporated to dryness in a water bath of 50°C and the residue was dissolved in 20 µl of HFBI heptafluorobutyrlimidazole, Pierce) in redistilled methylene chloride (HFBI: methylene chloride, 1:10) using a vortex-mixer. A 1-3 µl sample was taken for GLC analysis.

Tocainide concentrations in biological fluids were measured using a previously established calibration curve constructed by analysis of known amounts of tocainide added to normal blood or urine and plotting the peak height ratio (tocainide:internal standard) versus the tocainide concentration (see Figure 2.2). The coefficients of variation of the assay were as follows: 0.1 $\mu g.ml^{-1}$ (6.05%), 0.5 $\mu g.ml^{-1}$ (3.78%) 1.0 $\mu g.ml^{-1}$ (2.94%), 2.5 $\mu g.ml^{-1}$ (4.46%) and 5.0 $\mu g.ml^{-1}$ (3.12%) with n = 5 for each concentration. During determination of tocainide in biological samples in no case was a peak observed at the retention time of tocainide or of W36149 in blank samples.

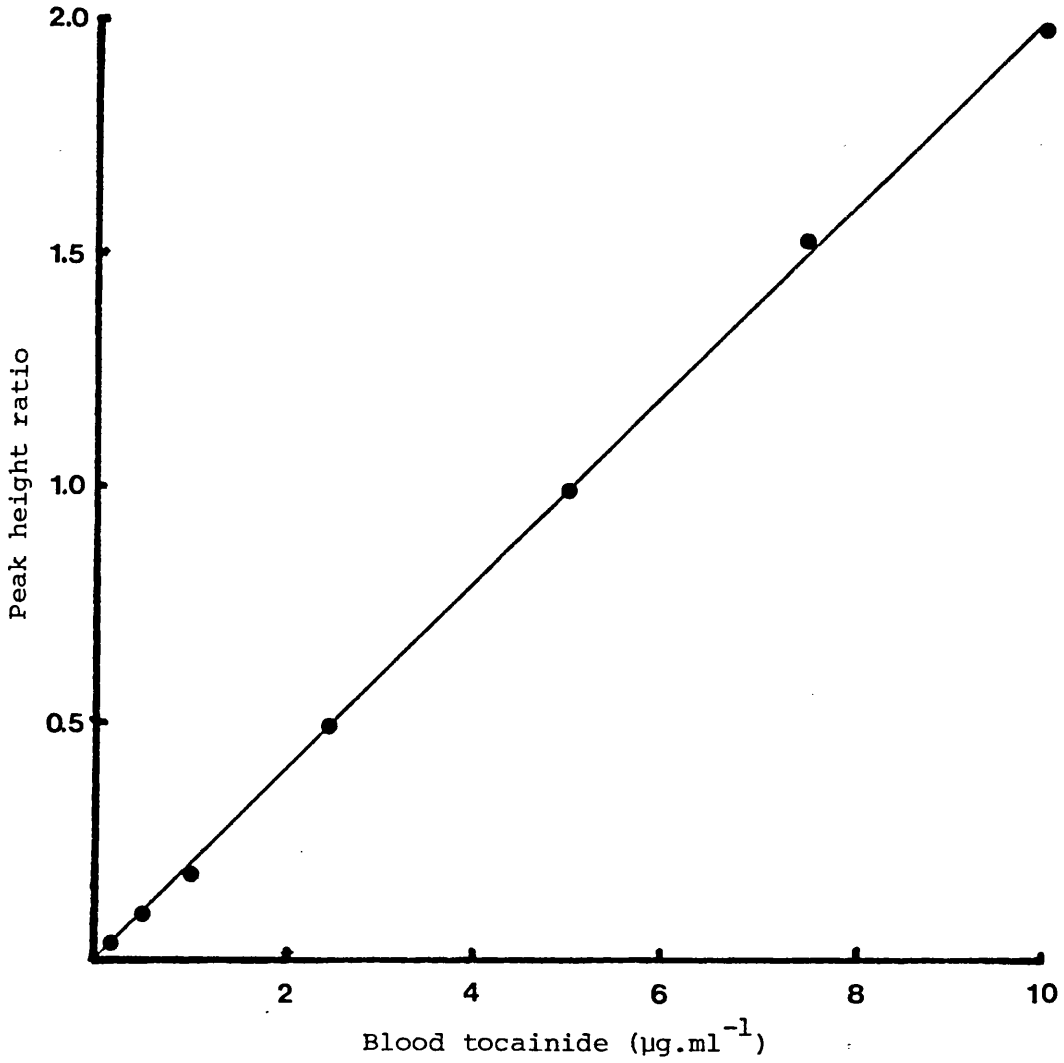



Figure 2.2 Standard calibration curve for tocainide in whole blood

## 2.8 Enzyme Induction parameters

Following the administration of the two enzyme inducing agents, phenobarbitone and 3,4-benzpyrene (see dosage and administration of compounds, 2.3) the following parameters of hepatic microsomal enzyme activity were measured:

i) Microsomal protein;

ii) Cytochrome P-450;

iii) p-nitroanisole O-demethylase activity;

iv) Pentobarbitone sleeping time.

### 2.8.1 Preparation of microsomes for enzyme assay

Twenty four hours after the last injection of phenobarbitone and 48 hours after 3,4-benzpyrene injection, rats which had been starved overnight were killed by exanguination from the carotid artery under ether anaesthesia. Each liver was immediately removed, rinsed in ice-cold phosphate buffer (pH7.25, see Appendix 22), blotted dry and weighed. Five to 10 g of liver was homogenised in ice-cold phosphate buffer (pH7.25) in a glass homogeniser with a motor driven teflon pestle. Homogenates were centrifuged at 10,000 g for 15 min at $4^{o}C$ in a M.S.E. Superspeed 65. The resulting supernatant was decanted without disturbing the pellet and centrifuged at 105,000g for 60 min at $4^{o}C$. After discarding the supernatant and rinsing the microsomal pellet with glycerol phosphate buffer (pH7.25, see Appendix 23), lipid adhering to the walls of the centrifuge tubes was wiped off with tissue paper. The microsomal pellet was resuspended in glycerol phosphate buffer (pH7.25, 2 ml of buffer for each gram of liver) with the aid of a teflon pestle. The microsomal suspension (approximately 10 mg protein $ml^{-1}$) was then stored at $-20^{o}C$ pending analysis.

2.8.2 Microsomal Protein Measurements

Microsomal protein concentration was determined in the SP 500 spectrophotometer by the method of Lowry et al (1951) using bovine serum albumin as standard. Figure 2.3 shows the standard curve for bovine serum albumin that was obtained using this method in the range 1-20 $mg.ml^{-1}$.

2.8.3 Cytochrome P-450 Assay

The content of cytochrome P-450 was determined by the method of Omura and Sato (1964).

Liver microsomes were diluted with glycerol phosphate buffer (pH7.25) such that 1 ml of the suspension contained 2-3 mg protein. $ml^{-1}$, then delivered to cuvettes of 1 cm light path, and 10 mg of sodium hydrosulfite (dithionite) were added to both sample and reference cuvettes. Carbon monoxide (Air Products Ltd., Surrey) was bubbled through the suspension in the sample cuvette for 30 sec. The difference in absorbance at 450 and 490 mμ (ΔE 450-490 mμ) was determined in a Unicam SP 1800 spectophotometer using a molar extinction coefficient of 91 $cm^{-1}$ $mM^{-1}$. Figure 2.4 shows carbon monoxide difference spectra of reduced microsomes for control and for 3,4-benzpyrene treated rats.

2.8.4 Para-nitroanisole O-demethylase assay

The microsomal O-demethylation of p-nitroanisole was determined by measuring the amount of formaldehyde which was produced during the demethylation of the substrate, p-nitroanisole to p-nitrophenol.

To 0.9 ml of incubation mixture (0.2 M Tris pH 7.2, 0.1 M $MgCl_2$) NADH and NADPH, see Appendix 24), 0.1 ml of microsomal suspension (approximately 10 mg. protein per ml) and 40 μl $1x10^{-3}$ M p-nitroanisole, 3.825 mg per ml methanol) were added, and maintained at 37°C in a

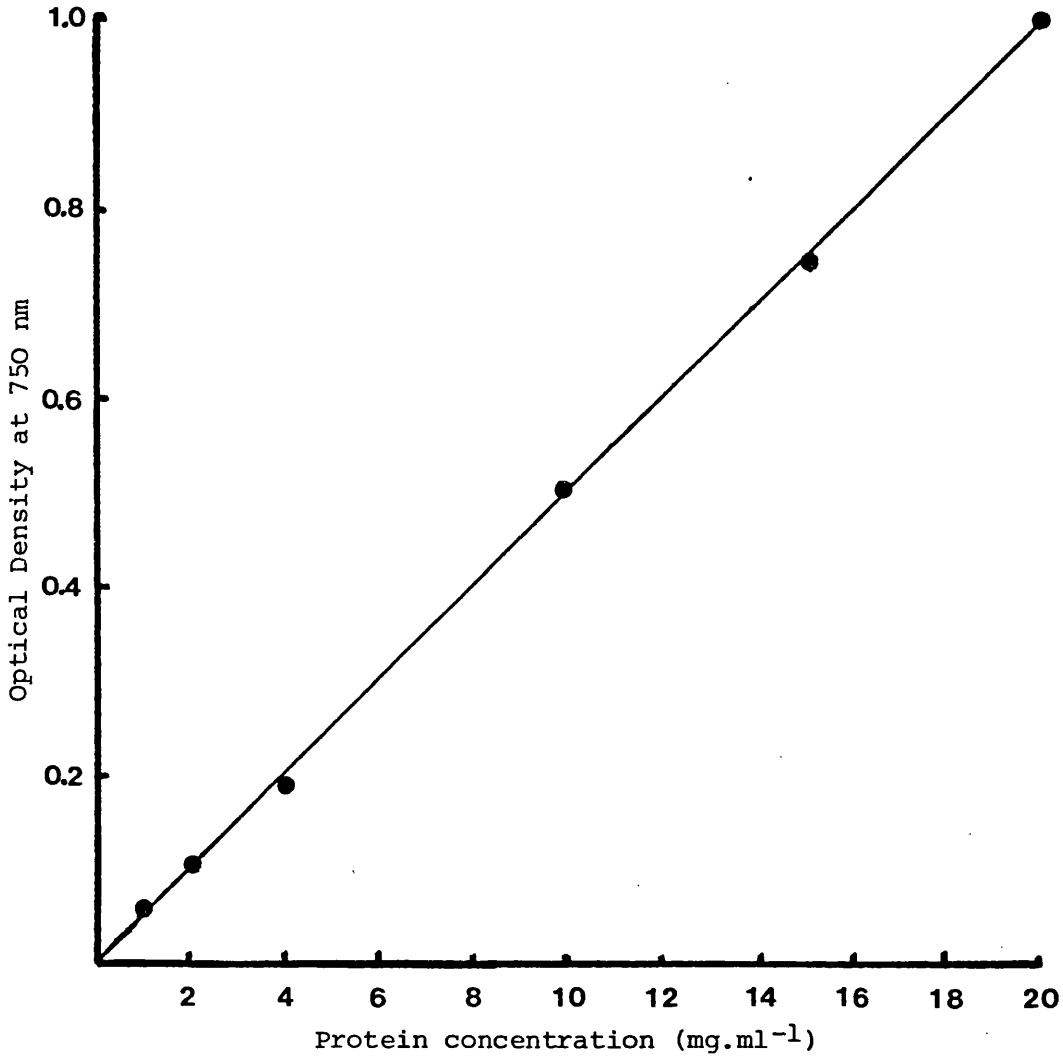



Figure 2.3 Standard curve for bovine serum albumin as used in the Lowry method for the estimation of protein concentration.

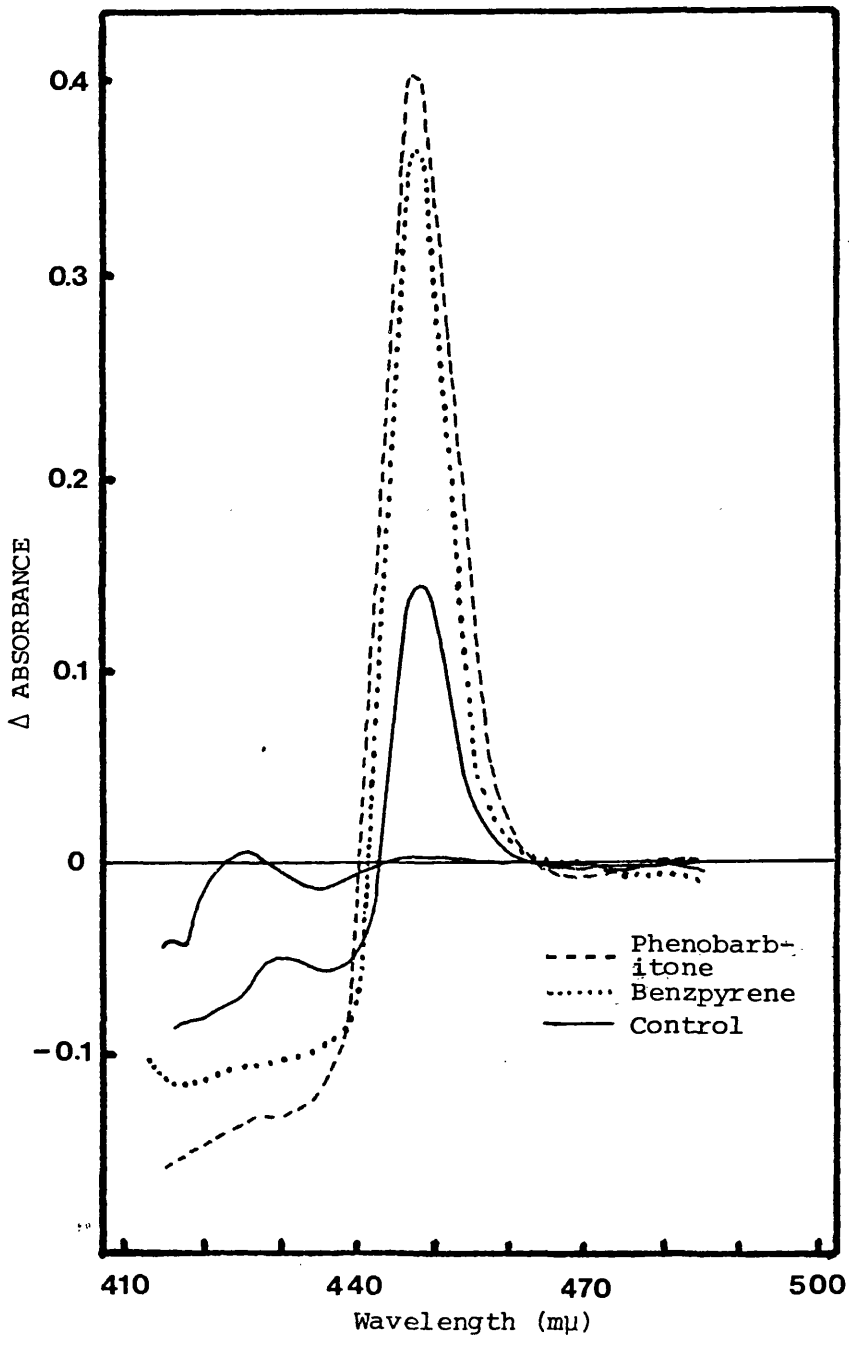



Figure 2.4 Carbon monoxide difference spectra of hepatic microsomes of control and treated rats.

Gallenkamp shaking water bath for 20 min. The reaction was stopped by the addition of 1 ml ice-cold 0.5 M citrate buffer (pH5.6, see Appendix 25). One ml of distilled water was added and the solution was transferred to a 15 ml stoppered glass tube and extracted three times with 5 ml of chloroform shaking for 60 sec per extraction. The organic phases were discarded and the aqueous phase was centrifuged at 1500 rpm for 15 min to precipitate protein. Two ml aliquots of the supernatant were assayed spectrophotometerically for the amount of formed formaldehyde according to the method of Nash (1953).

A standard curve for optical density plotted against formaldehyde concentration was constructed and is shown in Figure 2.5.

### 2.8.5 $V_{max}$ and $K_m$ determination

The maximum velocity ($V_{max}$) and Michaelis-Menten constant ($K_m$) values of p-nitroanisole in control and in induced microsomes were determined by using substrate concentrations of $0.25 \times 10^{-3}$M, $0.50 \times 10^{-3}$, $1.00 \times 10^{-3}$M and $1.50 \times 10^{-3}$M in the incubation mixture. The assay of p-nitroanisole O-demethylase activity was carried out as described above except that the reaction was stopped after 3,6,9,12 and 15 min.

$V_{max}$ and $K_m$ were obtained by plotting $\frac{1}{v}$ against $\frac{1}{s}$ (v = µmoles of formaldehyde formed per unit time and s = molar concentration of p-nitroanisole) $-\frac{1}{k_m}$ and $\frac{1}{V_{max}}$ were taken to be the x and y axis intercepts respectively.

### 2.8.6 Measurement of pentobarbitone sleeping time

Rats were given enzyme inducing agents as described above. Twenty-four hours after the last dose of phenobarbitone and 48 hours after 3,4-benzpyrene injection, pentobarbitone (40 $mg.kg^{-1}$) was given by i.p. injection. Sleeping time was defined as the time between loss and return of the righting reflex.

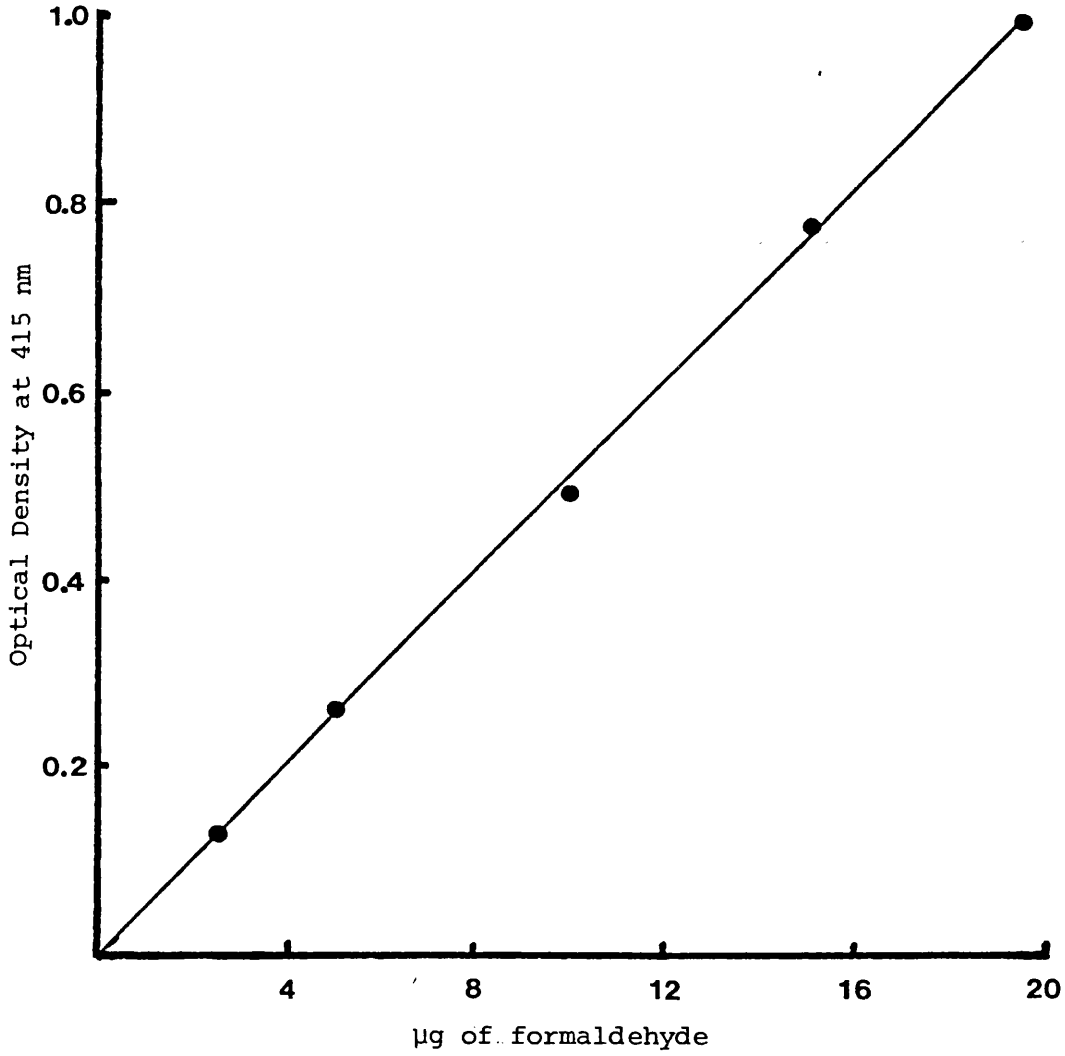



Figure 2.5 Standard curve for formaldehyde determination in the estimation of p-nitroanisole-O-demethylase.

## 2.9 Measurement of hepatic and organ blood flow

Hepatic and organ blood flow were determined by the method of McDevitt and Nies (1976) using radioactive microspheres. With this method the total cardiac output, its fractional distribution, and organ blood flow can be measured simultaneously in the same animal.

Rats were anaesthetized with sodium pentobarbitone (50 $mg.kg^{-1}$, i.p., Sagatal, May & Baker). The right carotid and right femoral arteries were cannulated with intravenous cannulae (outer diameter 1.02 mm and 0.75 mm respectively, Portex, Kent) and blood pressure was monitored from the left femoral artery using a Bell and Howell pressure transducer (type 4 - 4220001) connected to a two channel recorder (Devices M2). The arterial blood sample from the right femoral artery was withdrawn at a constant rate (0.6 ml $min^{-1}$) by a Perfusor IV pump (Braun, Melsungen, Germany). A third cannula was passed through the right carotid artery into the left ventricle, the placement being considered satisfactory only when a typical left ventricular pressure pulse tracing had been obtained from the pressure monitor (see Figure 2.6).

A vial containing 60-80,000 polystyrene microspheres (diameter ≃ 15 μm), labelled with $^{85}Sr$, in 0.6 ml 0.9% NaCl containing 0.02% Tween 80 at 37°C was placed in an ultrasonicator for at least 2 min to break down any aggregates of microspheres that may have formed. The solution thus mixed was drawn into a 1 ml syringe. After expulsion of air, the syringe was connected to the left ventricular cannula and the microspheres were injected over a 20 sec period and the cannula was rinsed with saline. For 70 sec after the injection of microspheres, arterial blood was withdrawn from the femoral artery at a rate of 0.6 ml $min^{-1}$. Systemic blood pressure was monitored during the microsphere injection to ensure that no alteration occurred

during this period. No change in pressure was noted in any animal used in this work. The rat was then killed with an overdose of sodium pentobarbitone and the placement of ventricular cannula was checked by visual examination of the opened thorax. Heart, lungs, liver, spleen, kidneys, gastrointestinal tract and pancreas were then excised and weighed.

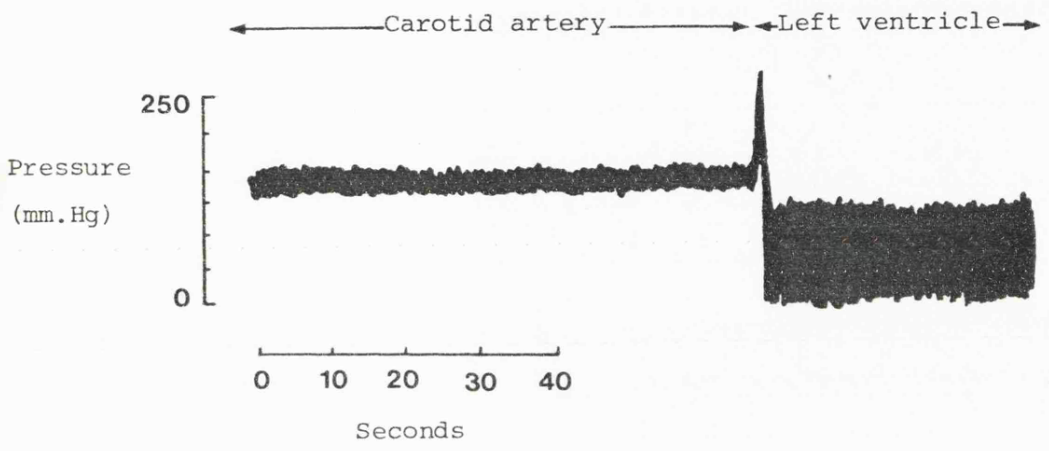



Figure 2.6 Change in arterial pressure in anaesthetized rat when a cannula was manipulated into the left ventricle through the carotid artery.

The excised organs were dissolved in warm concentrated nitric acid (30 ml for liver and gastrointestinal tract and 7 ml for the other organs) and adjusted to a volume of 100 ml (liver and GI tracts) or 25 ml (other organs) with 1% Tween 80 in $H_2O$.

Radioactivity in aliquots (4 ml) of these suspensions and in blood samples were measured in a NaI (Tl) Well-type Scintillation Counter (V.P. Engineering, Reading).

Cardiac output and organ blood flow were determined from the following formulae:

cardiac output =

$$\frac{\text{(total counts injected)x(blood sample withdrawal rate)}}{\text{(counts in blood sample)}}$$

and organ blood flow =

$$\frac{\text{(counts in organ)x(blood sample withdrawal rate)}}{\text{(counts in blood sample)}}$$

Because of its particular anatomical position the liver receives both hepatic arterial and portal venous blood. Hepatic arterial flow was determined from the microspheres trapped in the liver and portal venous return was obtained from microspheres trapped in spleen, pancreas and gastrointestinal tract (Nies et al, 1976). Liver blood flow, in this study, refers to the sum of hepatic arterial and portal venous flow.

## 2.10 Presentation of figures

For linear plots of drug concentration against time, a line was drawn to connect adjacent points.

For logarithmic plots of drug administered intravnously an arbitrary decision was made defining the initial rapid and the subsequent slow phase of decline of drug concentration. Lines were drawn to connect adjacent points in the rapid phase and the line was connected to the linear least square regression line for the points on the slow phase.

For logarithm plots of drug administered p.o. an arbitrary decision was made defining the absorption and the disposition phases of drug concentrations. Lines were drawn to connect adjacent concentration points in the absorption phase and the line was connected to the linear least squares regression line for the points on the disposition phase.

# CHAPTER III

## Pharmacokinetics of lignocaine and tocainide

3.1 Single intravenous doses of lignocaine

Twenty rats with body weights ranging between 370 g and 480 g were given lignocaine by intravenous bolus injection. Six rats received 2.5 $mg.kg^{-1}$, 8 rats received 5.0 $mg.kg^{-1}$ and 6 rats received 10.0 $mg.kg^{-1}$. Blood samples were taken before and after dosing at appropriate intervals and assayed for lignocaine. The blood concentration-time values for individual rats and the group means are given in Appendix 1 and the mean data are shown graphically on linear and on logarithmic scales plotted against time in Figure 3.1 and 3.2 respectively.

The mean concentrations of lignocaine in blood ranged from 0.19 to 3.16, 0.55 to 7.32 and 0.76 to 13.66 $\mu g.ml^{-1}$ following 2.5, 5.0 and 10.0 $mg.kg^{-1}$ respectively. AUC of lignocaine increased with the dose given. Concentration of lignocaine in blood declined in a biphasic manner with an initial rapid and a subsequent slow phase which was linear when logarithm of concentration is plotted against time (Figure 3.2). One rat, following a 2.5 $mg.kg^{-1}$ i.v. dose of lignocaine, exhibited a blood concentration-time curve with too short an $\alpha$ phase to permit estimation of A, $\alpha$ and $t_{\frac{1}{2}\alpha}$. The kinetic parameters of this rat were included in the mean data.

The kinetic parameters A, B, $\alpha$, $\beta$, $t_{\frac{1}{2}\alpha}$, $t_{\frac{1}{2}\beta}$, the rates constants ($k_{12}$, $k_{21}$ and $k_e$), the volume constant Vp, $Vd_{extrap}$, $Vd_{area}$, $Vd_{ss}$, AUC and the mean systemic clearance of lignocaine (total body clearance) were obtained from the blood concentration-time data for each animal using the non-linear least squares regression analysis programme NONLIN (Metzer, 1969) as described in Appendix 21.

Table 3.1 shows the mean data after single i.v. doses of lignocaine at three different doses. Using parametric (t) and non-parametric (Wilcoxon) statistical tests no significant differences in $\alpha$, $\beta$, Vp, $Vd_{extrap}$, $Vd_{area}$, $Vd_{ss}$ or in systemic clearance were found with change in dose.

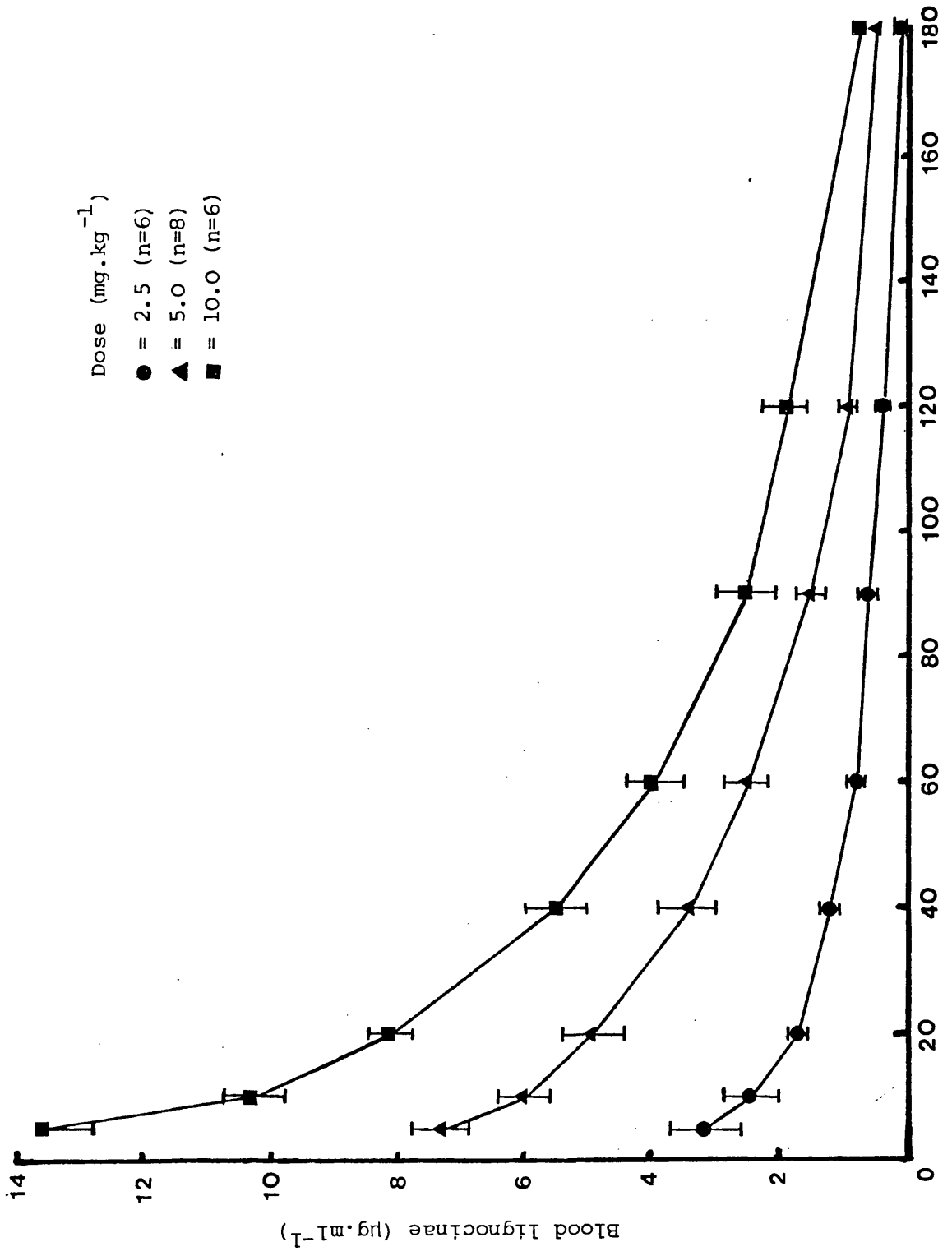



Figure 3.1 Mean (±S.E.M.) blood concentrations of lignocaine with respect to time after intravenous administration at doses of 2.5, 5.0 and 10.0 mg.kg$^{-1}$

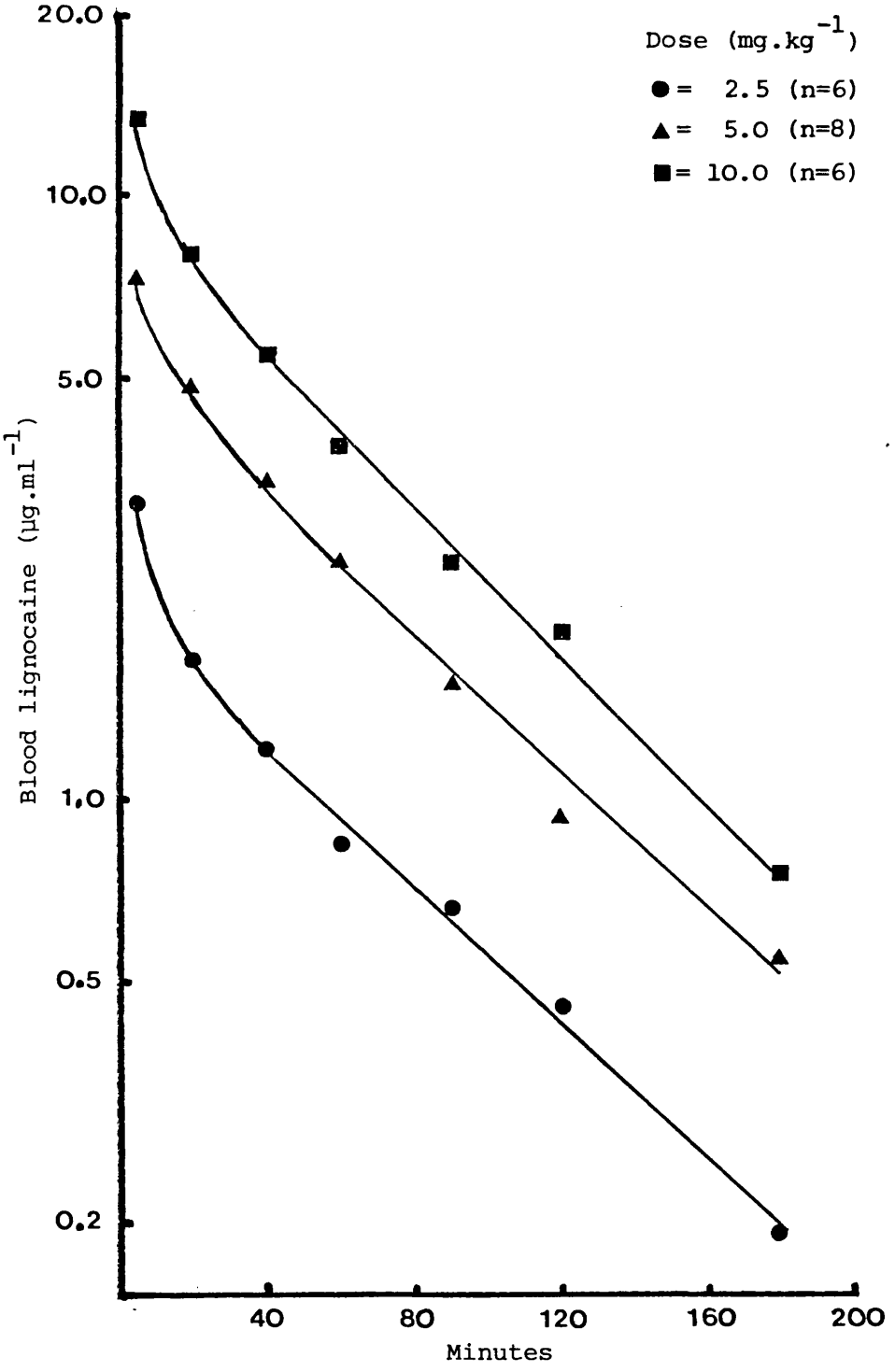



Figure 3.2 Mean blood concentrations of lignocaine with respect to time after intravenous injection of doses of 2.5, 5.0 and 10.0 mg.kg$^{-1}$

Table 3.1 Pharmacokinetic parameters of lignocaine following 2.5, 5.0 and 10.0 $mg.kg^{-1}$ by single intravenous injection. (Mean ± S.E.M.)

| Dose ($mg.kg^{-1}$) | n | Body weight (g) | A $\mu g.ml^{-1}$ | B $\mu g.ml^{-1}$ | $\alpha$ ($min^{-1}$) | $\beta$ ($min^{-1}$) | $t_{\frac{1}{2}\alpha}$ (min) | $t_{\frac{1}{2}\beta}$ (min) | Systemic clearance $ml.min^{-1}.kg^{-1}$ |
|---|---|---|---|---|---|---|---|---|---|
| 2.5 | 6 | 395 (±9.7) | 3.55 (±1.38) | 1.97 (±0.20) | 0.1721 (±0.0464) | 0.0126 (±0.0013) | 5.2 (±1.2) | 58.1 (±6.8) | 14.53 (±1.91) |
| 5.0 | 8 | 394 (±5.1) | 7.21 (±1.74 | 5.95 (±0.86) | 0.2208) (±0.0642) | 0.0148 (±0.0009) | 6.8 (±2.5) | 48.1 (±3.9) | 12.10 (±1.17) |
| 10.0 | 6 | 431 (±14.6) | 13.31 (±3.38) | 10.11 (±1.02) | 0.1201 (±0.0295) | 0.0139 (±0.0014) | 7.4 (±1.5) | 52.5 (±5.9) | 13.43 (±1.15) |

| Dose ($mg.kg^{-1}$) | n | Vp ($l.kg^{-1}$) | $Vd_{extrap}$ ($l.kg^{-1}$) | Vdarea ($l.kg^{-1}$) | $Vd_{ss}$ ($l.kg^{-1}$) | $k_{12}$ ($min^{-1}$) | $k_{21}$ ($min^{-1}$) | $k_e$ ($min^{-1}$) |
|---|---|---|---|---|---|---|---|---|
| 2.5 | 6 | 0.62 (±0.15) | 1.34 (±0.17) | 1.17 (±0.14) | 1.11 (±0.14) | 0.0775 (±0.0319) | 0.0780 (±0.0220) | 0.0283 (±0.0061) |
| 5.0 | 8 | 0.48 (±0.07) | 1.01 (±0.19) | 0.84 (±0.10) | 0.76 (±0.08) | 0.1119 (±0.0417) | 0.1101 (±0.0252) | 0.0275 (±0.0034) |
| 10.0 | 6 | 0.59 (±0.07) | 1.18 (±0.10) | 0.92 (±0.10) | 0.89 (±0.06) | 0.0428 (±0.0160) | 0.0664 (±0.0128) | 0.0249 (±0.0033) |

The mean values of AUC of lignocaine are given in Table 3.2. Figure 3.3 shows the relationship between AUC and dose, over the dose range used. The areas under the blood concentration time curves of lignocaine after intravenous administration are linearly related to dose ($r = 0.883$, $p<0.001$) and the 95% confidence interval for the slope of the regression line includes the origin of the plot of AUC against dose.

Table 3.2. Mean (± SEM) values for area under blood-concentration-time curves of lignocaine after intravenous administration at doses of 2.5, 5.0 and 10.0 $mg.kg^{-1}$.

| Dose ($mg.kg^{-1}$) | 2.5 | 5.0 | 10.0 |
|---|---|---|---|
| AUC ($\mu g.ml^{-1}.min$) | 188.03 ±25.01 | 444.72 ±48.19 | 774.32 ±64.05 |
| n | 6 | 8 | 6 |

Figure 3.4 shows dose-normalised blood-concentration time curves for lignocaine over the range studied and indicates that the curves may be superimposed.

The constancy of the above pharmacokinetic parameters with changing dose of lignocaine indicates that for the rat in the dose-range studied the kinetics are dose-independent. Accordingly when alteration in the physiological determinants of lignocaine disposition were studied (Chapter 4,5) the middle dose (5.0 $mg.kg^{-1}$) was selected.

Recovery of lignocaine in the urine after a dose of 2.5 $mg.kg^{-1}$, i.v., was estimated in two rats and found to be less than 3% of the administered dose. No sign of toxicity was observed in the rats over the dose range studied.

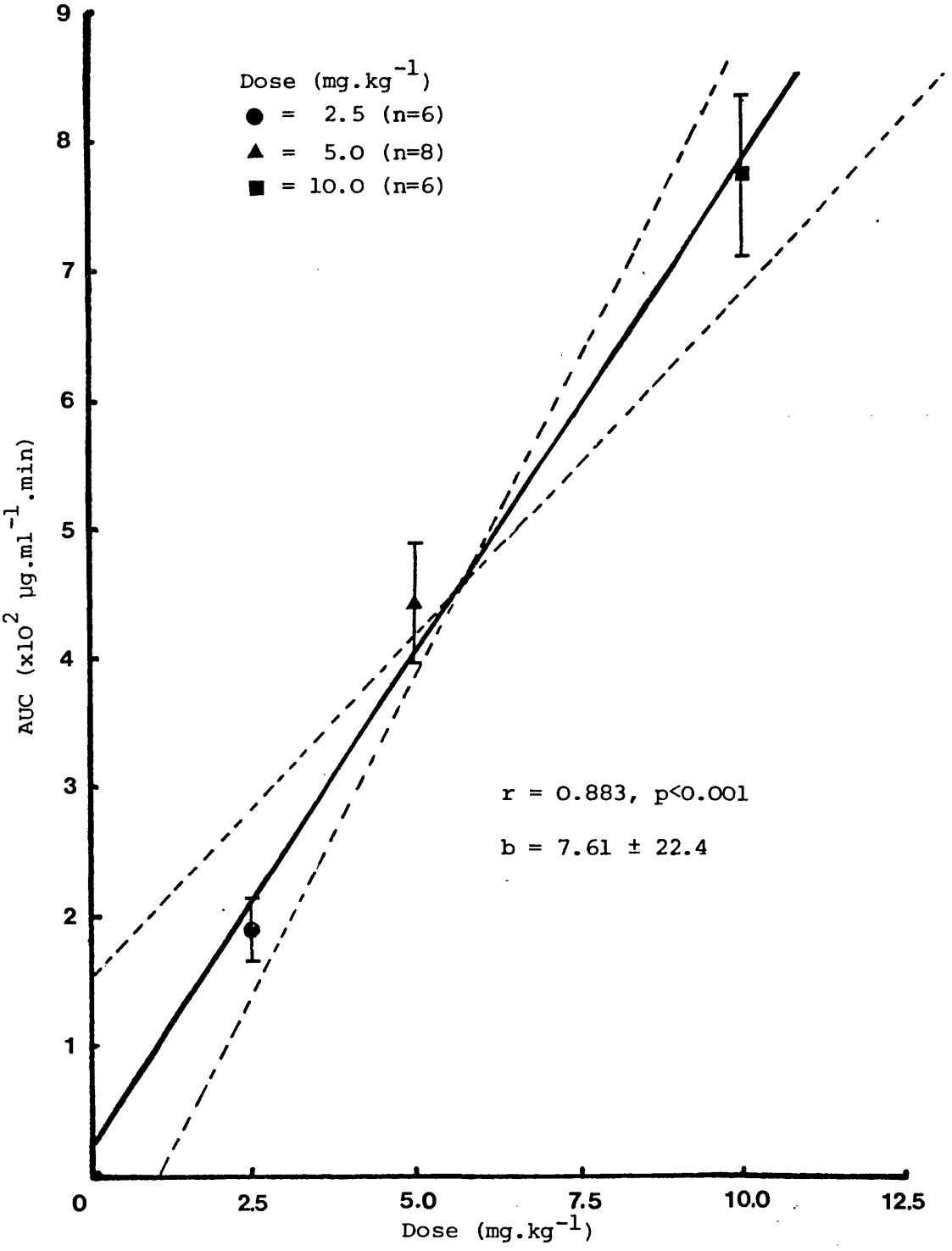



Figure 3.3 Relationship between AUC and dose following intravenous administration of lignocaine. (Mean ± S.E.M.)

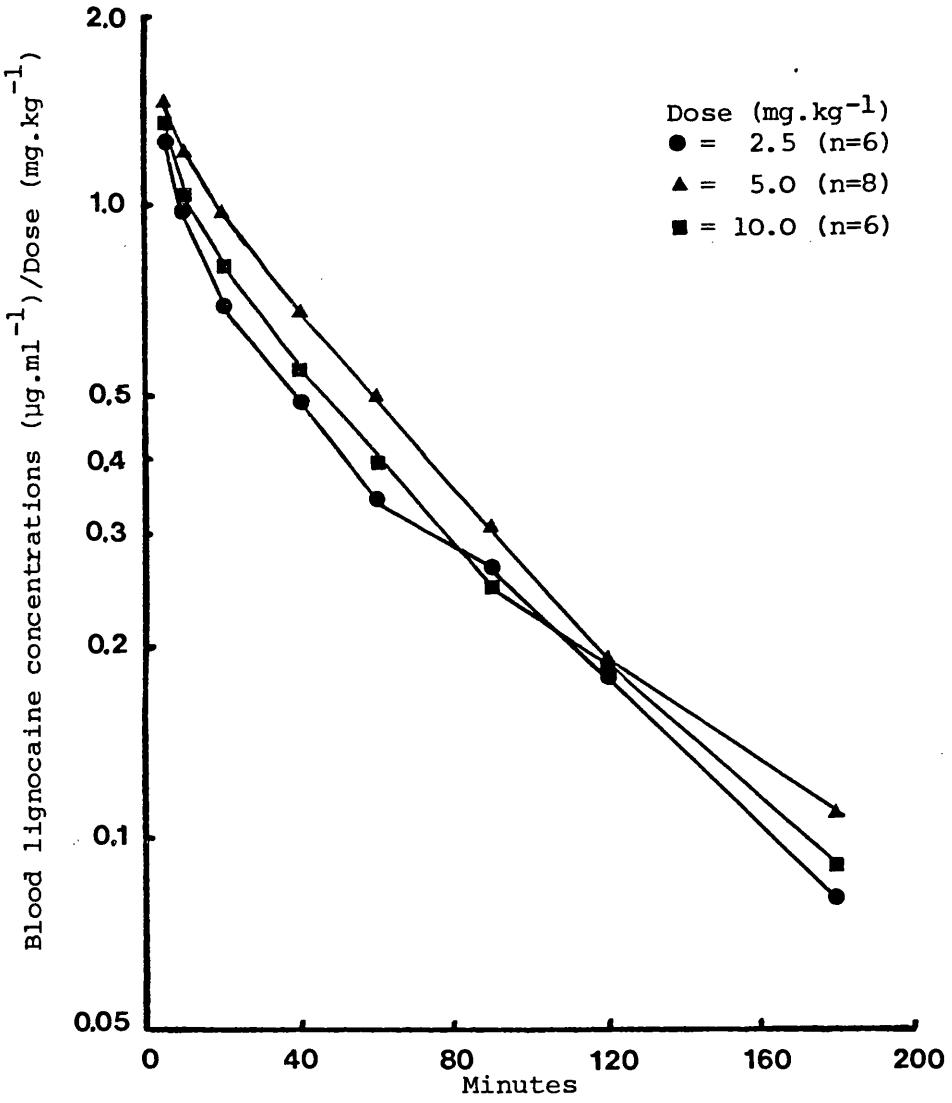



Figure 3.4 Superposition plot of mean blood lignocaine concentrations with respect to time following intravenous doses of 2.5, 5.0 and 10 mg.kg$^{-1}$.

## 3.2 Single oral doses of lignocaine

Twenty-four rats with body weights ranging between 355 g to 475 g were given lignocaine by mouth. Eight rats received 50 $mg.kg^{-1}$, eight received 70 $mg.kg^{-1}$ and eight received 90 $mg.kg^{-1}$. Blood samples were taken before and after dosing and assayed for lignocaine. The blood concentration-time data for individual rats and the group means appear in Appendix 3. The mean blood concentrations ranged from 0.13 to 0.60, 0.13 to 0.88 and 0.29 to 1.29 $\mu g.ml^{-1}$ at the 50, 70 and 90 $mg.kg^{-1}$ dose levels respectively. Lignocaine was detected in the blood within 10 min and the maximum concentration occurred 30-45 min after drug administration.

Figures 3.5 and 3.6 show plots of mean blood concentration of lignocaine on a linear and on a logarithmic scale respectively against time. It is apparent that after single oral administration the AUC increases with the dose given and that the terminal phase of the plot of logarithm of drug concentration with respect to time is linear.

The kinetic parameters $k_{ab}$, $k_d$, $t_{\frac{1}{2}}$, AUC and oral clearance were obtained from the blood concentration-time data for each animal as demonstrated in Appendix 20 and the mean data appear in Table 3.3. There was no significant difference in $k_e$ and consequently $t_{\frac{1}{2}}$ did not change with dose.

Table 3.4 presents the mean values for the AUC and these values are also plotted against dose in Figure 3.7. This shows that AUC is linearly related to dose over the range studied ($r = 0.872$, $p<0.001$). It is noted that the regression line does not pass through the origin, rather it crosses the x-axis at a dose equivalent to 23 $mg.kg^{-1}$. However, the 95% confidence interval for the slope of the regression line does include the origin of the plot of AUC against dose.

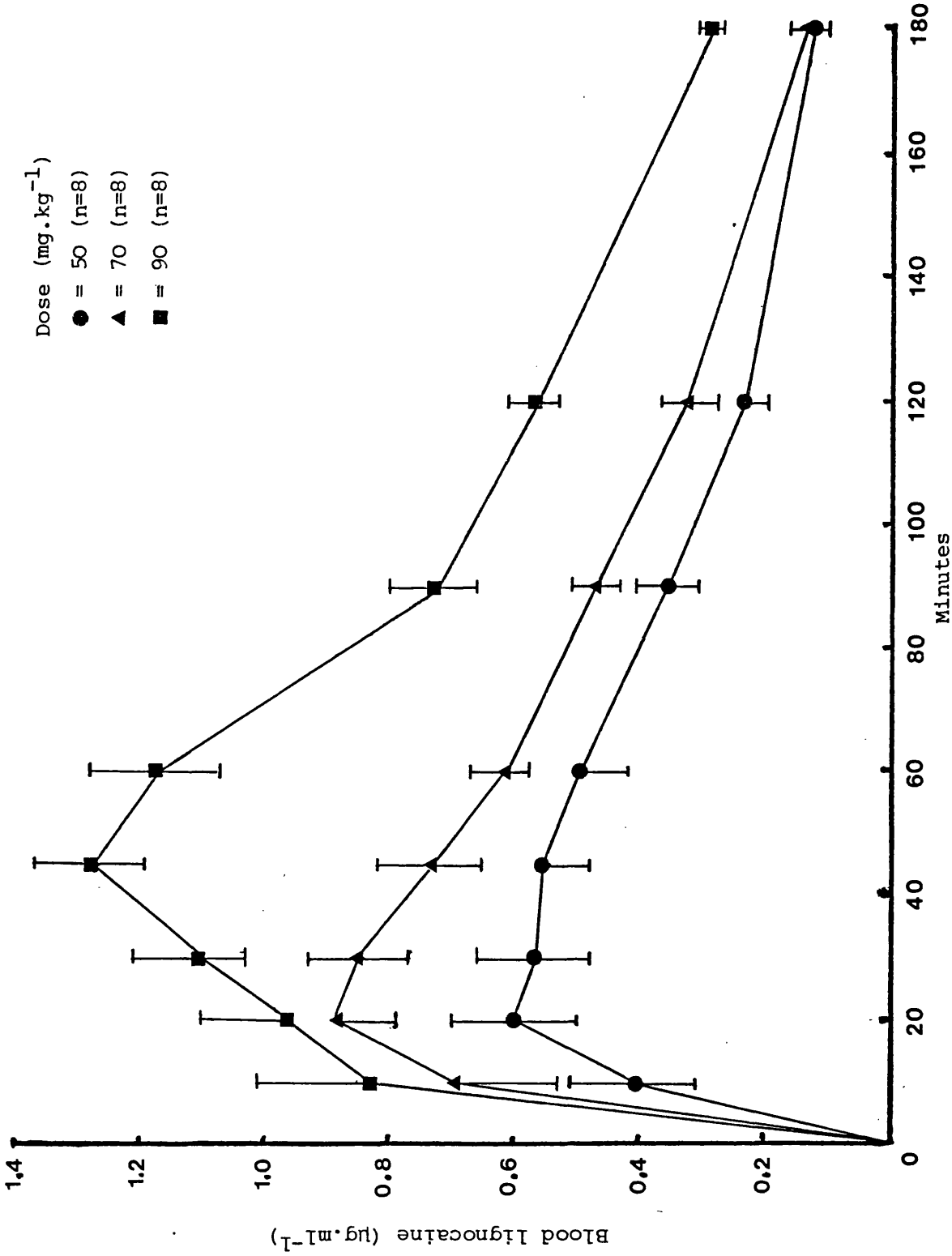



Figure 3.5 Mean (±S.E.M.) blood concentrations of lignocaine with respect to time after oral administration of 50, 70 and 90 mg.kg$^{-1}$

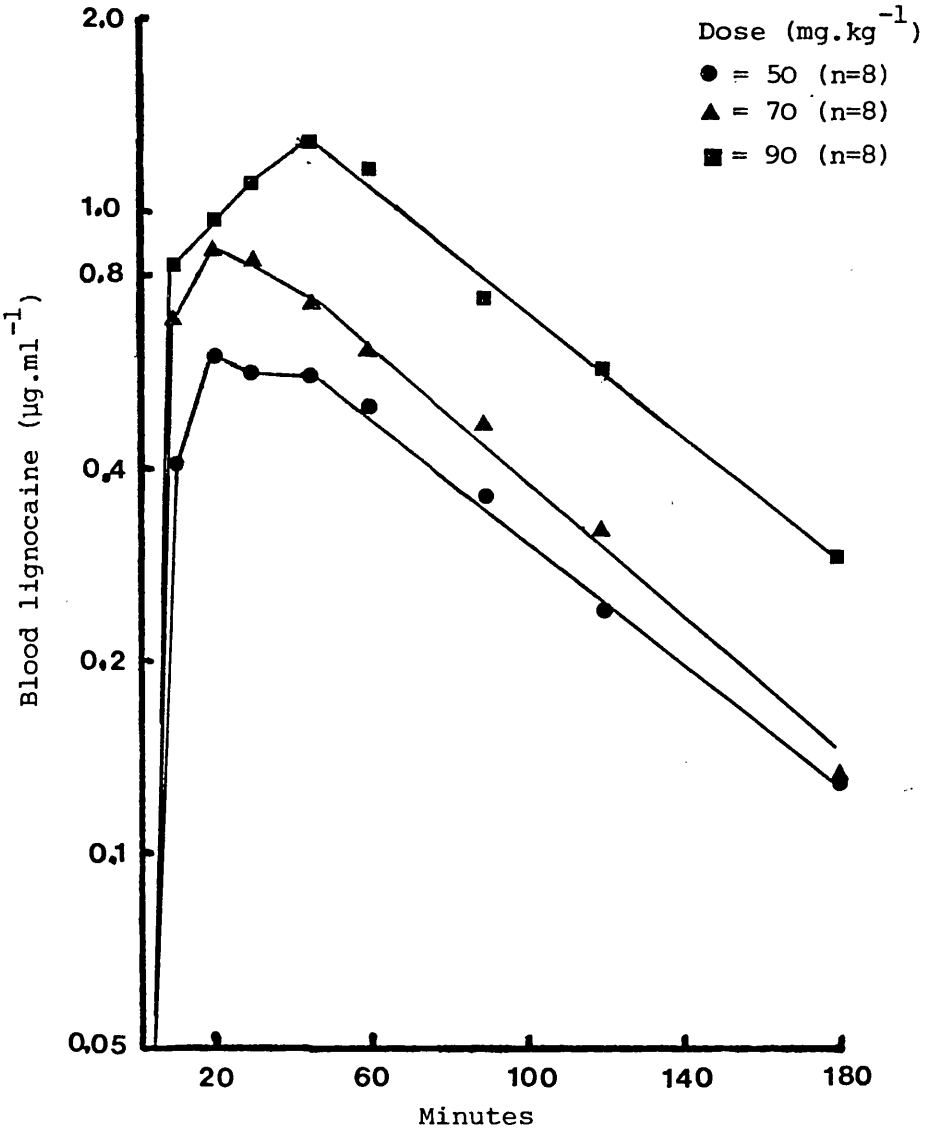



Figure 3.6 Mean blood concentrations of lignocaine with respect to time following oral doses of 50, 70 and 90 mg.kg$^{-1}$

Table 3.3 Kinetic parameters of lignocaine HCl following oral administration of doses of 50, 70 and 90 $mg.kg^{-1}$ (Mean ± SEM)

| Dose ($mg.kg^{-1}$) | 50.0 | 70.0 | 90.0 |
|---|---|---|---|
| n | 8 | 8 | 8 |
| Body weight (g) | 423.1±11.8 | 417.5±13.2 | 405.0±8.5 |
| $k_{ab}$ ($min^{-1}$) | 0.0484±0.0084 | 0.1044±0.0174 | 0.0654±0.0085 |
| $k_d$ ($min^{-1}$) | 0.0124±0.0012 | 0.0121±0.0009 | 0.0112±0.0007 |
| $t_{\frac{1}{2}}$ (min) | 60.1±6.5 | 59.2±4.6 | 63.1±3.9 |
| Apparent clearance (oral) ($ml.min^{-1}.kg^{-1}$ | 865.6±154.8 | 729.7±79.8 | 565.1±19.9 |

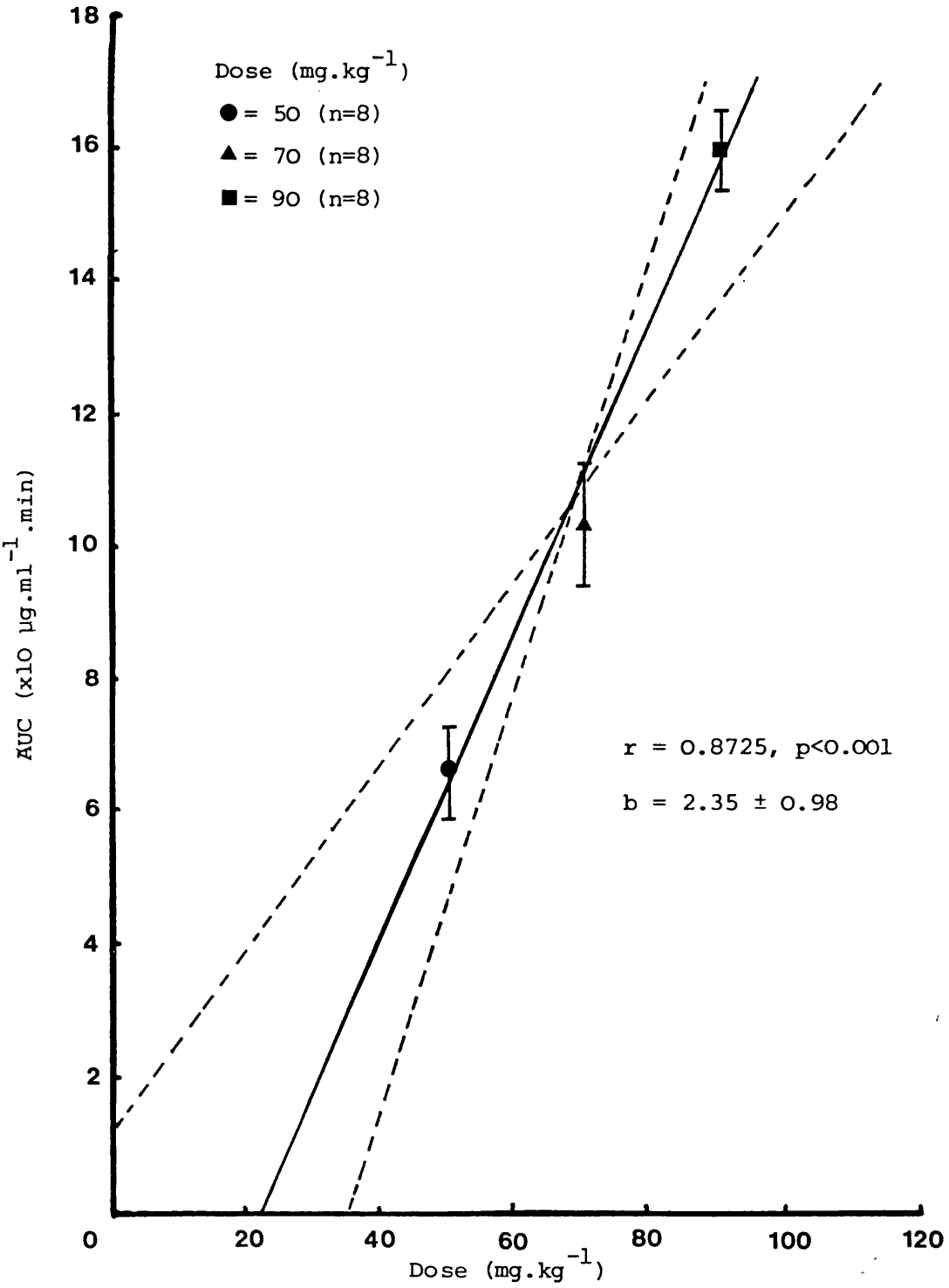



Figure 3.7 Relationship between AUC and dose for lignocaine following oral administration (Means ± S.E.M.)

Table 3.4 Mean (±SEM) values for area under the blood concentration-time curves after oral administration of lignocaine at doses of 50, 70 and 90 $mg.kg^{-1}$.

| Dose ($mg.kg^{-1}$) | 50.0 | 70.0 | 90.0 |
|---|---|---|---|
| AUC ($\mu g.ml^{-1}.min$) | 66.68 ±7.78 | 103.10 ±9.48 | 160.72 ±5.96 |
| n | 8 | 8 | 8 |

Figure 3.8 shows dose normalised blood concentration-time curves for lignocaine for the doses studied and indicates that the curves are superposable. Apparent clearance of lignocaine after oral administration fell with increasing dose but the differences were not significant.

Systemic availability of orally administered lignocaine, calculated as the ratio of the dose normalised AUC at each oral dose to the mean of all dose normalised AUC's obtained after i.v. administration, is shown in Table 3.5. No statistically significant differences were found indicating the dose-independency of orally administered lignocaine within the range studied.

After oral administration, the amount of drug recovered as unchanged lignocaine in urine was found to be less than 1%. Rats receiving lignocaine at a dose of 90 $mg.kg^{-1}$ exhibited reduced activity but no other adverse effect of lignocaine were noted.

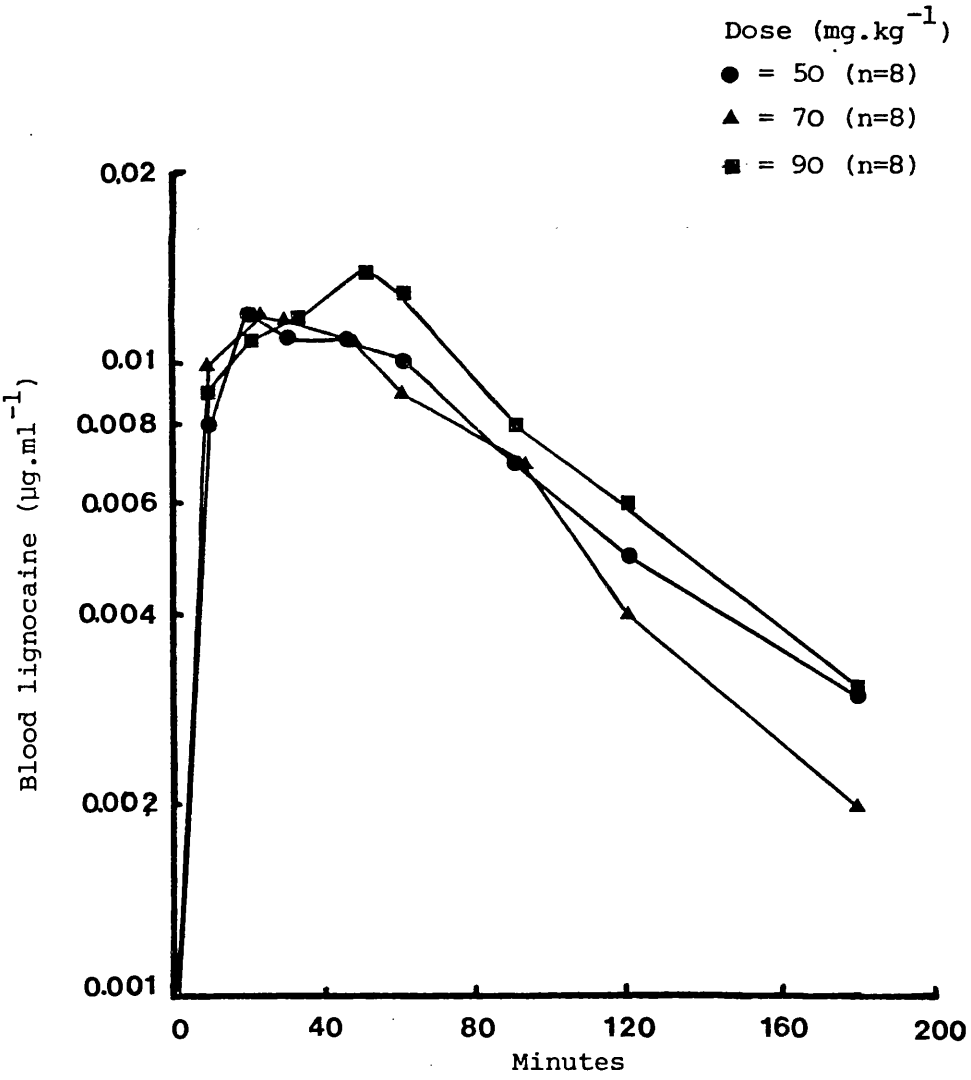



Figure 3.8 Superposition plot of mean blood lignocaine concentrations with respect to time following oral administration of 50, 70 and 90 mg.kg$^{-1}$

Table 3.5 Systemic availability of lignocaine following oral administration at doses of 50.0, 70.0 and 90.0 $mg.kg^{-1}$.

| Oral Dose ($mg.kg^{-1}$) | Mean $AUC_{p.o.}$ ($\mu g.ml^{-1}.min$) | Normalized Mean $AUC_{i.v.}$ ($\mu g.ml^{-1}.min$) | Availability |
|---|---|---|---|
| 50 | 66.68 | 4026.50 | 0.0166 |
| 70 | 103.10 | 5637.10 | 0.0183 |
| 90 | 160.70 | 7247.70 | 0.0222 |
| Mean | — | — | 0.0190±0.0016 |

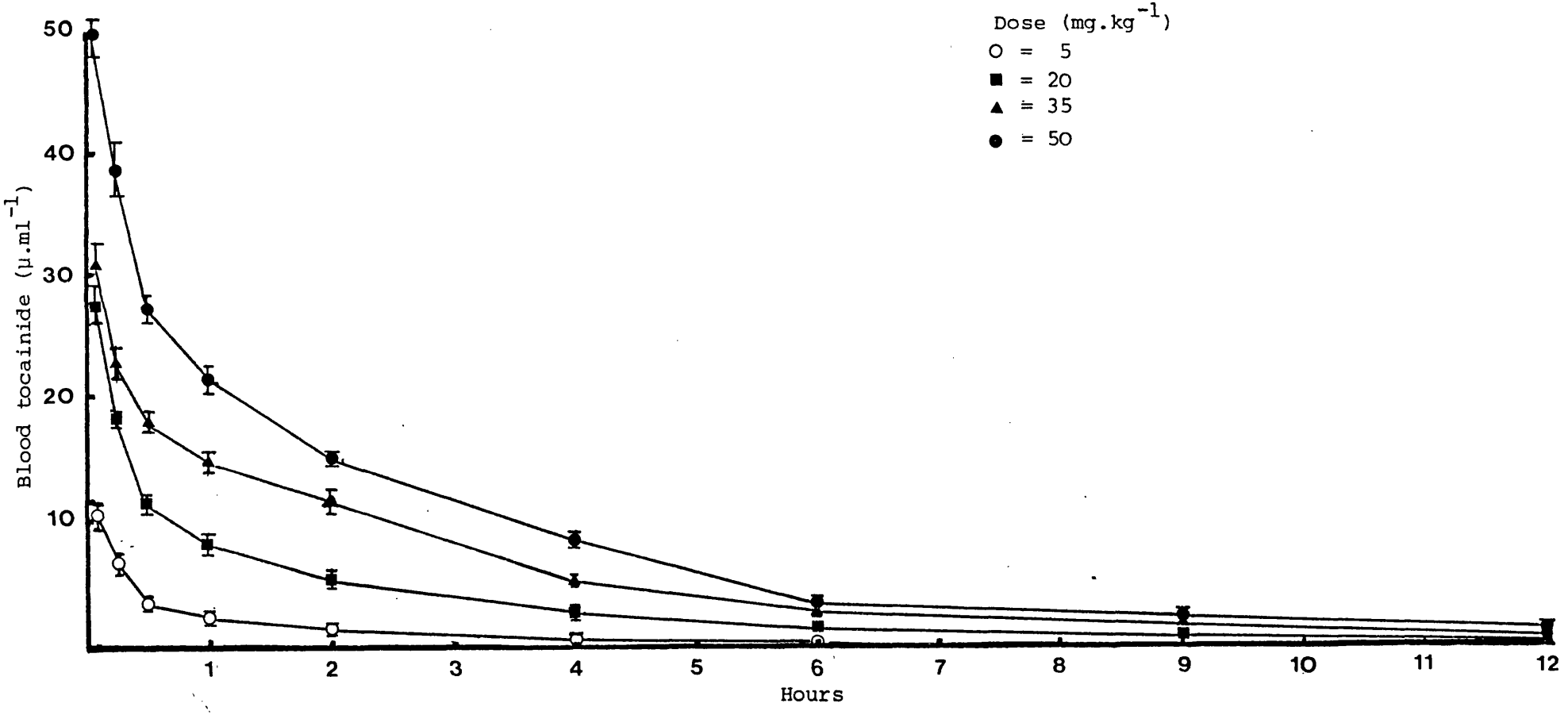
3.3 Single intravenous doses of tocainide

The duration of restraint in metabolic cages necessiated by this study was longer than that required for the experiments on lignocaine and attempts to collect both blood and urine simultaneously after dosing with tocainide were unsuccessful. The experiments were therefore designed in such a way that each animal received two doses of tocainide on separate occasions; after one dose urine only was collected and after the other dose blood only was collected.

Tocainide was given by i.v. bolus injection to rats in doses of 5, 20, 35 and 50 $mg.kg^{-1}$. Five rats were studied at each dose making a total of 20 rats (body weight 465-575 g). The rats were kept in metabolic cages and all urine passed in the 24 hr after dosing was collected. One week later each animal again received the same dose of tocainide by i.v. bolus injection and blood was taken at appropriate times and assayed for tocainide.

The blood tocainide concentration-time data for individual animals and the group means are given in Appendix 5. The mean blood concentrations of tocainide plotted against time at the four doses are shown graphically on a linear scale in Figure 3.9 and on a logarithmic scale in Figure 3.10.

The mean concentrations of tocainide ranged between 0.21 and 10.21, 0.29 and 27.45, 0.54 and 30.61 and 0.93 and 49.49 $\mu g.ml^{-1}$ after doses of 5, 20, 35 and 50 $mg.kg^{-1}$, respectively. After intravenous injection the concentration of tocainide in the blood declined in biphasic manner with an initial rapid and a subsequent slow phase which was linear when the logarithm of concentration was plotted against time (Figure 3.10). Kinetic parameters were obtained from the blood concentration-time data for each animal using the non-linear least squares regression analysis programme NONLIN as described in Appendix 21 and the mean values for these parameters at the four doses appear in Table 3.6.




Firgure 3.9 Mean (±S.E.M.) blood concentrations of tocainide hydrochloride following single intravenous doses of 5, 20, 35 and 50 mg.kg$^{-1}$. Five rats were studied at each dose level.

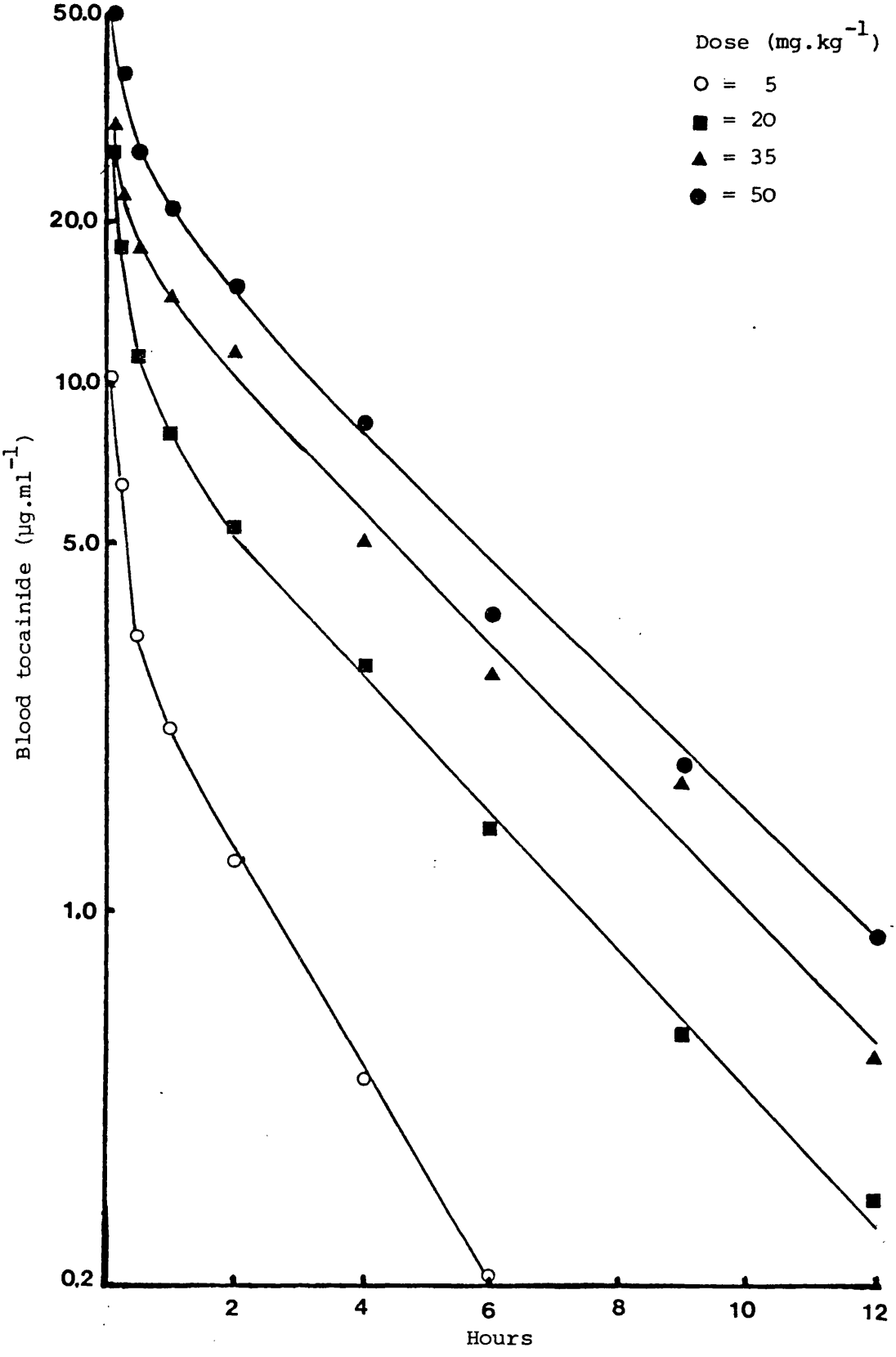



Figure 3.10 Mean blood concentration of tocainide with respect to time following intravenous administration of doses of 5, 20, 35 and 50 mg.kg$^{-1}$. Five rats were studied at each dose level

Table 3.6 Pharmacokinetic parameters of tocainide following single intravenous doses of 5, 20, 35 and 50 $mg.kg^{-1}$. (Mean ± S.E.M.)

| Dose ($mg.kg^{-1}$) | n | Body weight (g) | A ($\mu g.ml^{-1}$) | B ($\mu g.ml^{-1}$) | $\alpha$ ($min^{-1}$) | $\beta$ ($min^{-1}$) | $t_{\frac{1}{2}\alpha}$ (min) | $t_{\frac{1}{2}\beta}$ (min) |
|---|---|---|---|---|---|---|---|---|
| 5 | 5 | 481.0 ± 5.8 | 11.66 ±1.01 | 3.07 ±0.89 | 0.1037 ±0.0326 | 0.0069 ±0.0011 | 8.7 ±1.6 | 113.4 ±18.4 |
| 20 | 5 | 488.0 ± 5.1 | 25.56 ±3.66 | 10.37 ±0.76 | 0.0727 ±0.0074 | 0.0053 ±0.0002 | 10.0 ±1.3 | 130.5 ±4.6 |
| 35 | 5 | 518.0 ±15.9 | 23.49 ±9.57 | 20.77 ±0.95 | 0.1219 ±0.0325 | 0.0054 ±0.0002 | 7.0 ±1.3 | 127.9 ±4.9 |
| 50 | 5 | 511.0 ±14.9 | 32.27 ±1.04 | 27.37 ±0.32 | 0.0680 ±0.0089 | 0.0051 ±0.0002 | 10.8 ±1.3 | 136.6 ±4.3 |

| Dose ($mg.kg^{-1}$) | n | Vp ($l.kg^{-1}$) | $Vd_{extrap}$ ($l.kg^{-1}$) | $Vd_{area}$ ($l.kg^{-1}$) | $Vd_{ss}$ ($l.kg^{-1}$) | $k_{12}$ ($min^{-1}$) | $k_{21}$ ($min^{-1}$) | $k_e$ ($min^{-1}$) |
|---|---|---|---|---|---|---|---|---|
| 5 | 5 | 0.35* ±0.03 | 2.13 ±0.48 | 1.49 ±0.27 | 1.06 ±0.16 | 0.0539 ±0.0195 | 0.0307 ±0.0134 | 0.0260 ±0.0011 |
| 20 | 5 | 0.58* ±0.06 | 1.97 ±0.16 | 1.66 ±0.10 | 1.41 ±0.06 | 0.0372 ±0.0054 | 0.0247 ±0.0018 | 0.0161 ±0.0022 |
| 35 | 5 | 0.89 ±0.13 | 1.70 ±0.07 | 1.62 ±0.06 | 1.55 ±0.05 | 0.0585 ±0.0276 | 0.0573 ±0.0037 | 0.0115 ±0.0030 |
| 50 | 5 | 0.84 ±0.01 | 1.83 ±0.02 | 1.67 ±0.03 | 1.54 ±0.04 | 0.0287 ±0.0044 | 0.0342 ±0.0045 | 0.0101 ±0.0002 |

* $p<0.02$

The data for AUC following different intravenous doses of tocainide appear in Table 3.7.

Table 3.7 Values for areas under blood concentration-time curves of tocainide following single intravenous injection at four doses to rat.

(Mean ± SEM)

| Dose (mg.kg) | 5 | 20 | 35 | 50 |
|---|---|---|---|---|
| AUC ($\mu g.ml^{-1}.min$) | 570.83 ±28.48 | 2293.38 ±116.69 | 4001.46 ±196.85 | 5901.95 ±168.74 |
| n | 5 | 5 | 5 | 5 |

Dose independent kinetics are indicated by the linear increase in AUC with dose (Figure 3.11). Dose normalized blood concentration-time curves for the doses studied are shown in Figure 3.12 and are superimposable a finding indicative of dose-independent kinetics.

Accordingly a dose of 35 $mg.kg^{-1}$, which was in the middle of the range studied, was selected for the multiple dose study (3.7) and also the studies in which blood flow and drug metabolism were altered (Chapter 4, 5). Total body clearance and renal clearance of tocainide following intravenous dosing were calculated for each rat as indicated in Appendix 20 and the mean values are listed in Table 3.8. No significant difference in total body clearance was found for the four doses studied (range of means 8.50-8.85 $ml.min^{-1}.kg^{-1}$). The percentage of total body clearance accounted for by non-renal clearance was also constant within the dose range studied (77.6-82.1%).

Symptoms of toxicity did not appear in rats receiving i.v. tocainide in the range of 5 to 35 $mg.kg^{-1}$. Sedation occurred in four out of five rats with a dose of 50 $mg.kg^{-1}$ but no serious toxic effects were noted.

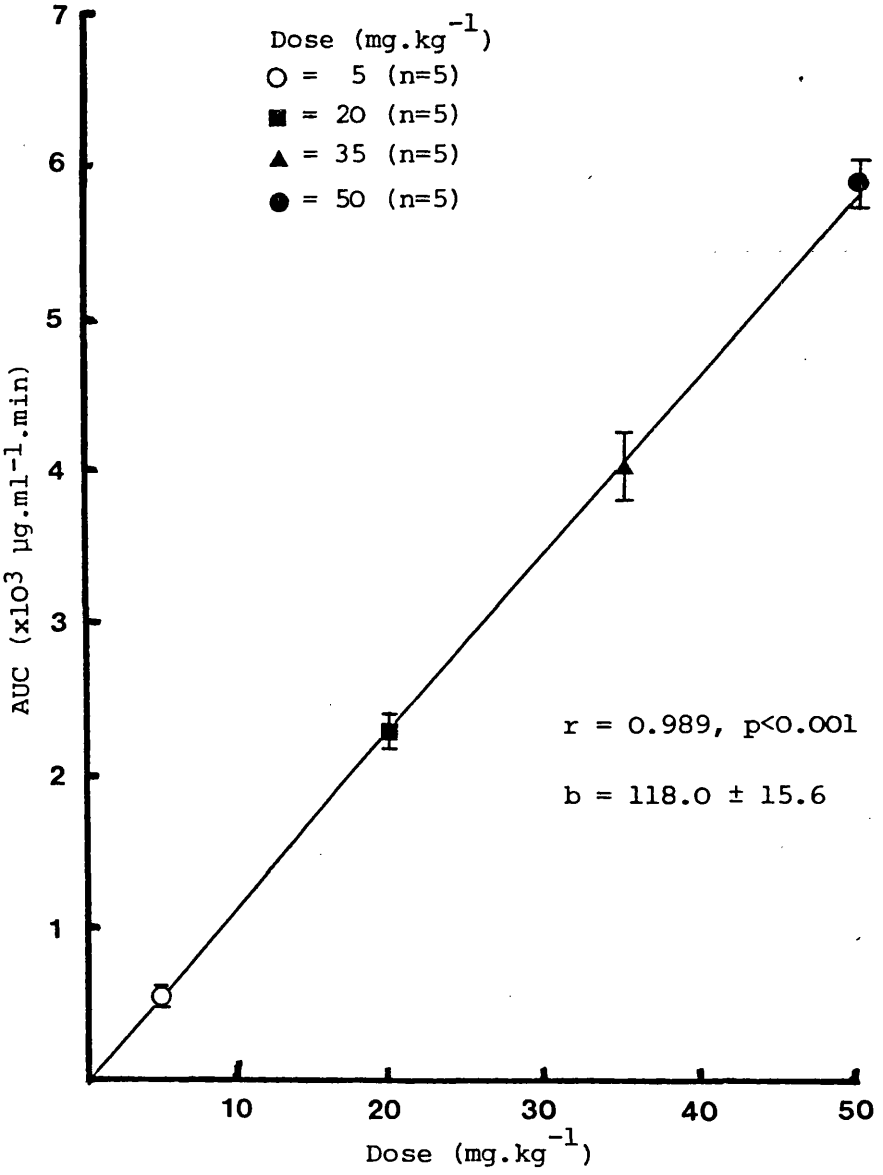



Figure 3.11 Relationship between AUC and dose following intravenous administration of tocainide (mean ± S.E.M.)

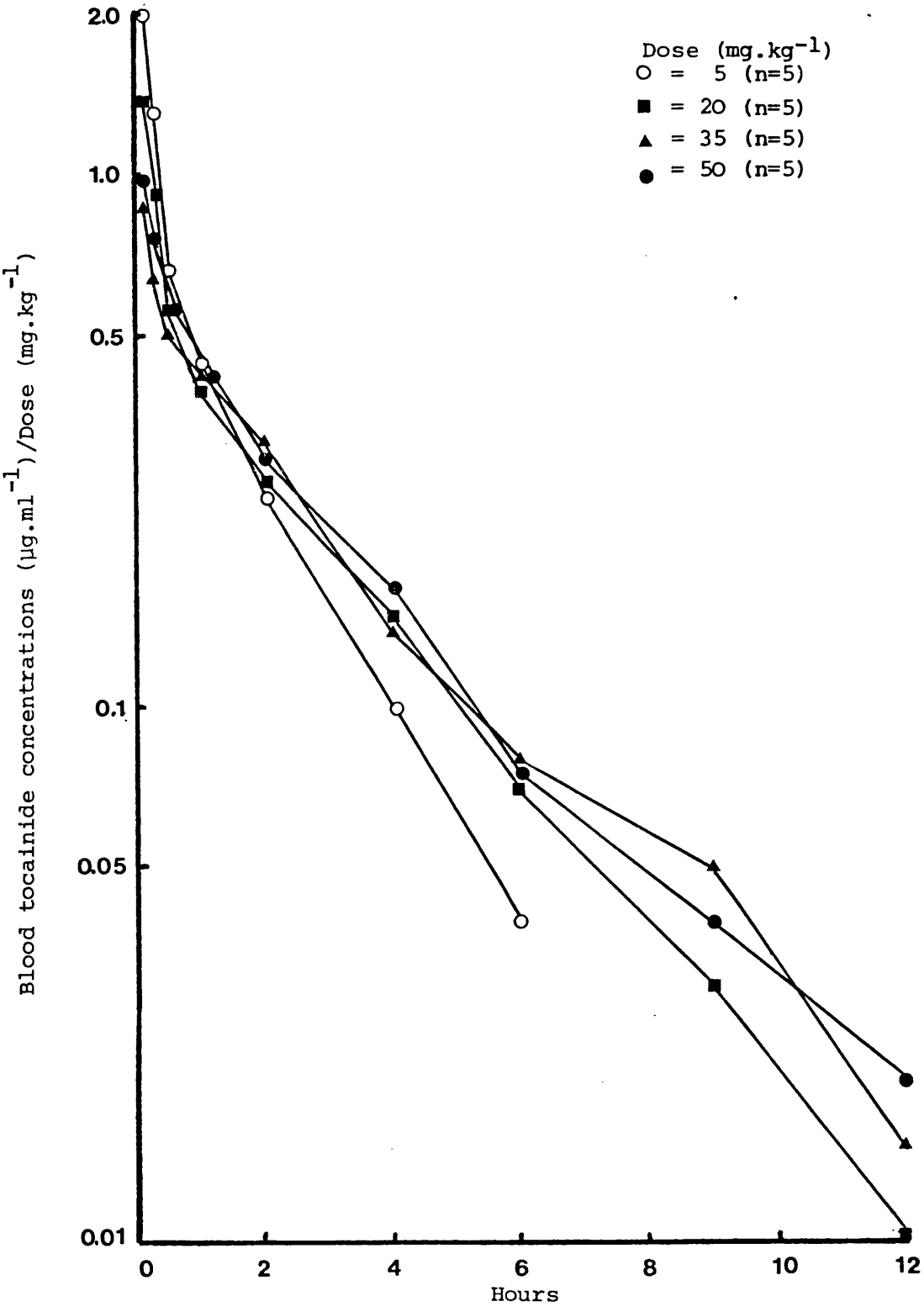



Figure 3.12 Superposition plot of the means of blood tocainide concentration ($\mu g.ml^{-1}$) following four intravenous doses

Table 3.8 Total body clearance, renal and nonrenal clearance of tocainide following single intravenous doses (Mean ± S.E.M.)

| Dose (mg.kg) | n | Total Body Clearance | | Renal Clearance | | Non-renal Clearance | | |
|---|---|---|---|---|---|---|---|---|
| | | $ml.min^{-1}$ | $ml.min^{-1}.kg^{-1}$ | $ml.min^{-1}$ | $ml.min^{-1}.kg^{-1}$ | $ml.min^{-1}$ | $ml.min^{-1}.kg^{-1}$ | % of total CL |
| 5 | 5 | 4.26±0.26 | 8.85±0.48 | 0.95±0.11 | 1.98±0.24 | 3.31±0.22 | 6.86±0.42 | 77.63±2.50 |
| 20 | 5 | 4.30±0.25 | 8.82±0.48 | 0.95±0.13 | 1.95±0.27 | 3.34±0.22 | 6.83±0.40 | 77.81±2.60 |
| 35 | 5 | 4.56±0.21 | 8.83±0.43 | 0.87±0.13 | 1.71±0.27 | 3.69±0.33 | 7.12±0.60 | 80.32±3.28 |
| 50 | 5 | 4.35±0.21 | 8.50±0.25 | 0.77±0.06 | 1.46±0.17 | 3.58±0.22 | 6.98±0.25 | 82.14±1.81 |

## 3.4 Single oral doses of tocainide

Twenty-two rats with body weights ranging between 450 and 575 g were given tocainide by mouth in doses of 50, 100, 200, 300 and 400 $mg.kg^{-1}$. Blood samples were taken at appropriate times and assayed for tocainide. The individual blood concentration-time data and the mean for each dose group appear in Appendix 7. Tocainide mean blood concentrations at each dose ranged as follows; 0.21-7.15 $\mu g.ml^{-1}$ (50 $mg.kg^{-1}$), 0.89-12.16 $\mu g.ml^{-1}$ (100 $mg.kg^{-1}$), 2.09-15.85 $\mu g.ml^{-1}$ (200 $mg.kg^{-1}$), 183-28.05 $\mu g.ml^{-1}$ (300 $mg.kg^{-1}$) and 2.08-54.59 $\mu g.ml^{-1}$ (400 $mg.kg^{-1}$). Maximum blood concentrations were reached between 2.5 and 4 hours after dosing. The mean blood concentration data are plotted in Figure 3.13 on a linear scale and in Figure 3.14 on a logarithmic scale with respect to time. It is apparent that the plot of logarithm of drug concentrations with respect to time is linear and that AUC increased with the dose given (Table 3.9).

The kinetic parameters $k_{ab}$, $k_d$, $t_{\frac{1}{2}}$ and CL were calculated and the mean values appear in Table 3.9. At the 50 $mg.kg^{-1}$ dose, $t_{\frac{1}{2}}$ was significantly shorter by student's 't' test ($p<0.001$) than the $t_{\frac{1}{2}}$ at all other doses. The relationship between $t_{\frac{1}{2}}$ and dose is depicted graphically in Figure 3.15. It is also noted that the $t_{\frac{1}{2}}$ of tocainide after oral administration is substantially longer (4.6-11.7 hr) than that obtained after the drug was given intravenously (113.4-130.5 min).

Within the range studied dose increased linearly with AUC ($r = 0.976$, $p<0.001$) and the 95% confidence interval for the slope of the regression line includes the origin of the plot of AUC against dose (Figure 3.16). Dose-normalized blood concentration-time curves for the doses studied are shown in Figures 3.17. Between doses of 100 to 400 $mg.kg^{-1}$ the plots are superimposable.

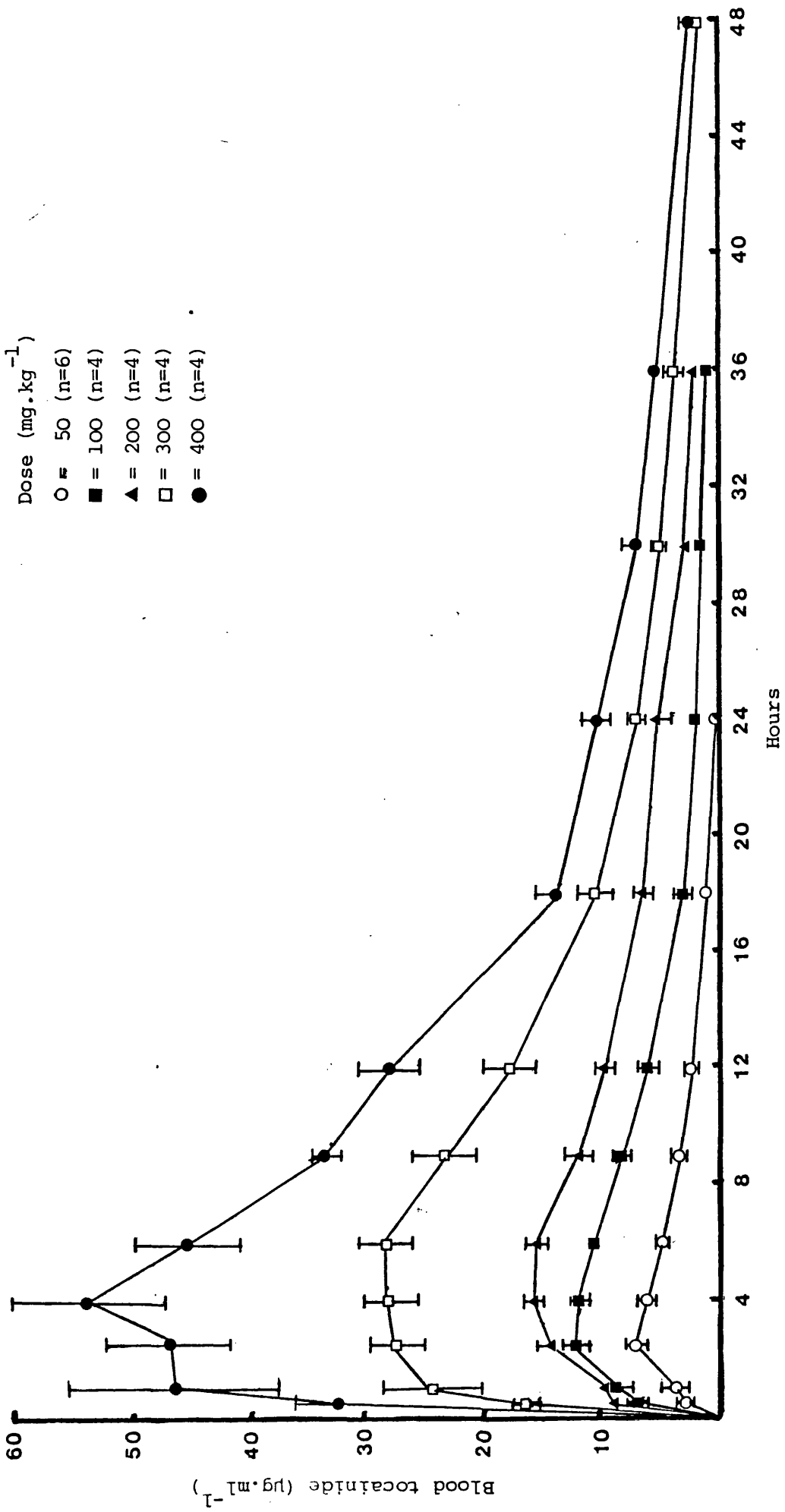



Figure 3.13 Mean (±S.E.M.) blood concentrations of tocainide HCl following oral administration

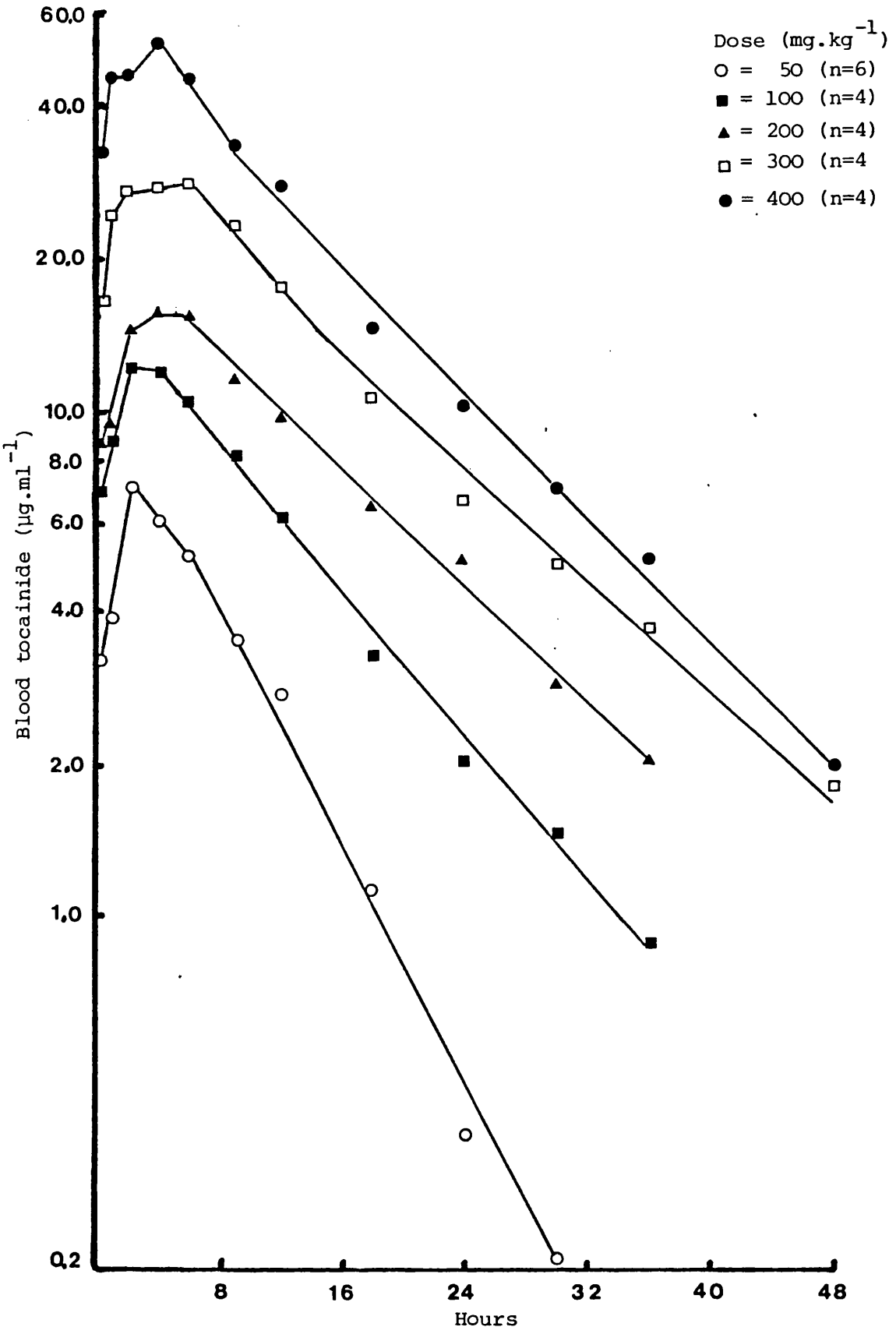



Figure 3.14 Mean blood concentrations of tocainide with respect to time after oral administration

Table 3.9 Kinetic parameters of tocainide hydrochloride following oral administration. (Mean ± S.E.M.)

| Dose ($mg.kg^{-1}$) | n | Body Weight (g) | $k_{ab}$ ($min^{-1}$) | $k_d$ ($min^{-1}$) | $t_{\frac{1}{2}}$ (hrs) | Clearance ($ml.min^{-1}$) | Clearance ($ml.min^{-1}.kg^{-1}$) | $AUC_{0\rightarrow\infty}$ $\mu g.ml^{-1}.min$ |
|---|---|---|---|---|---|---|---|---|
| 50.0 | 6 | 524.2 ±14.2 | 0.0090 ±0.0023 | 0.0026 ±0.0002 | 4.6±0.3** | 6.8±1.2 | 13.0±2.3 | 4303.0±544.3 |
| 100.0 | 4 | 495.0 ±6.4 | 0.0122* ±0.0046 | 0.0013 ±0.0002 | 8.3±0.2 | 4.5±0.2 | 8.9±0.4 | 11043.5±465.7 |
| 200.0 | 4 | 490.0 ±10.8 | 0.0112 ±0.0035 | 0.0011 ±0.0001 | 11.1±1.3 | 5.3±0.3 | 10.9±0.7 | 18530.4±1266.4 |
| 300.0 | 4 | 503.7 ±9.0 | 0.0059 ±0.0008 | 0.0010 ±0.0001 | 11.7±1.6 | 7.7±0.3 | 9.3±0.6 | 32675.0±1972.5 |
| 400.0 | 4 | 521.2 ±10.9 | 0.0068 ±0.0033 | 0.0012 ±0.0001 | 10.0±1.2 | 4.2±0.2 | 8.1±0.5 | 50007.5±2954.8 |

* n = 3

** $p<0.001$

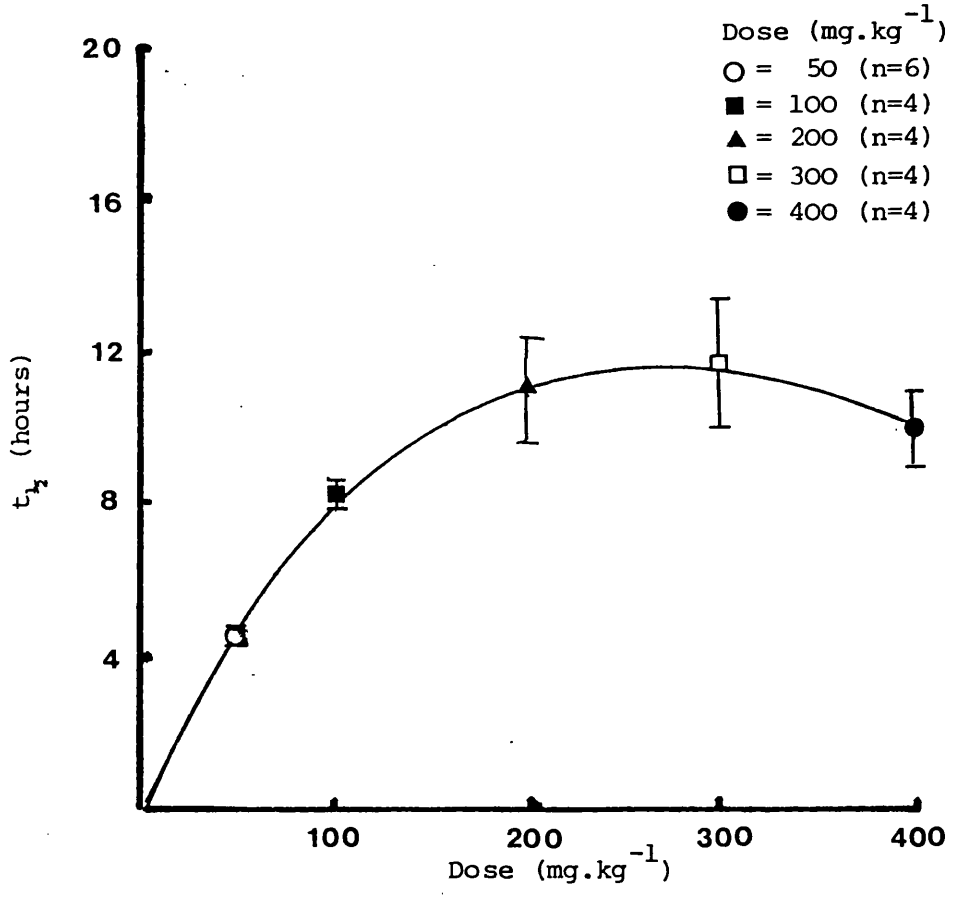



Figure 3.15 $T_{\frac{1}{2}}$ with respect to dose of tocainide following oral administration. (Mean ± S.E.M.)

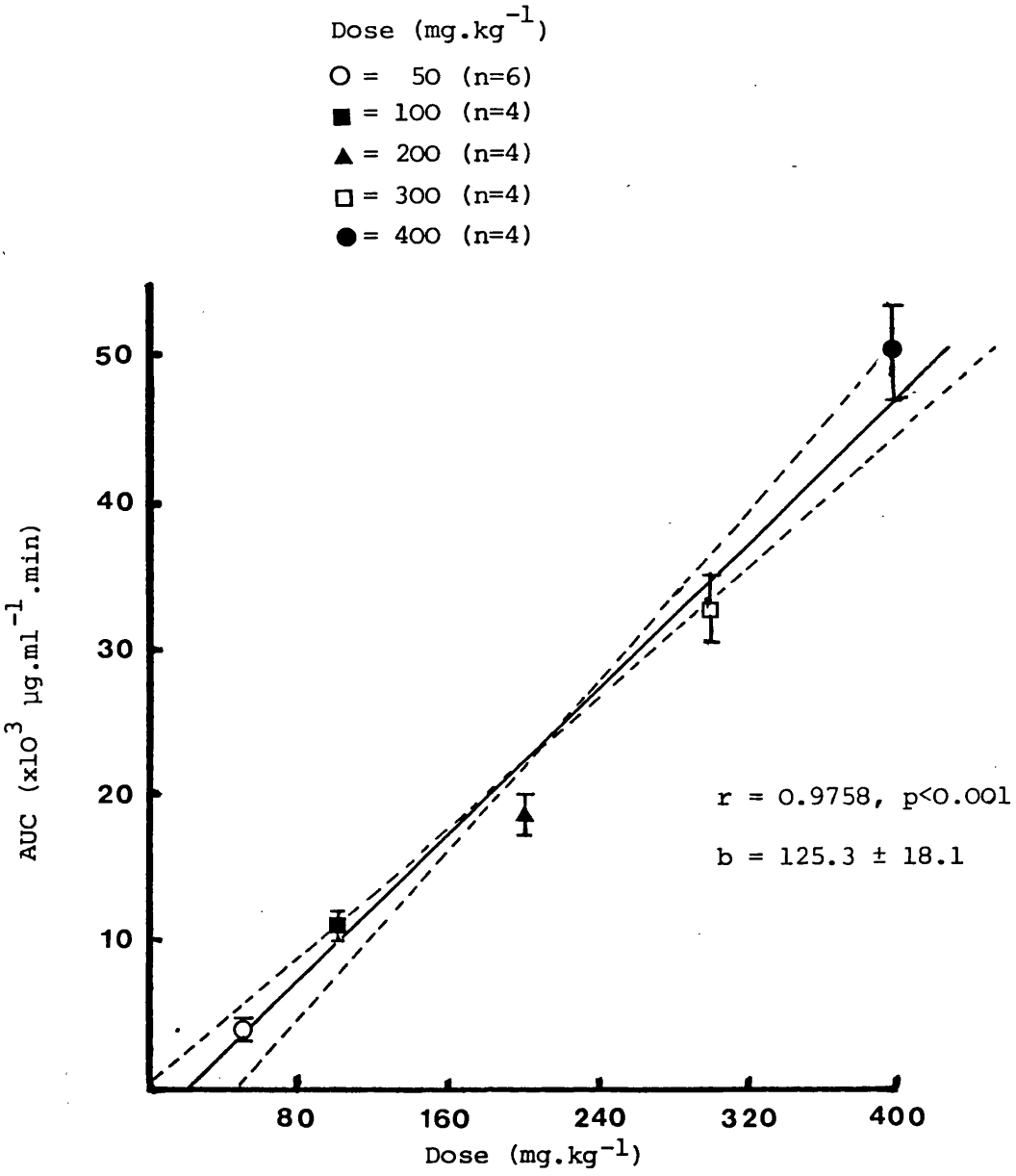



Figure 3.16 Relationship between AUC and dose following oral administration of tocainide. (Mean ± S.E.M.)

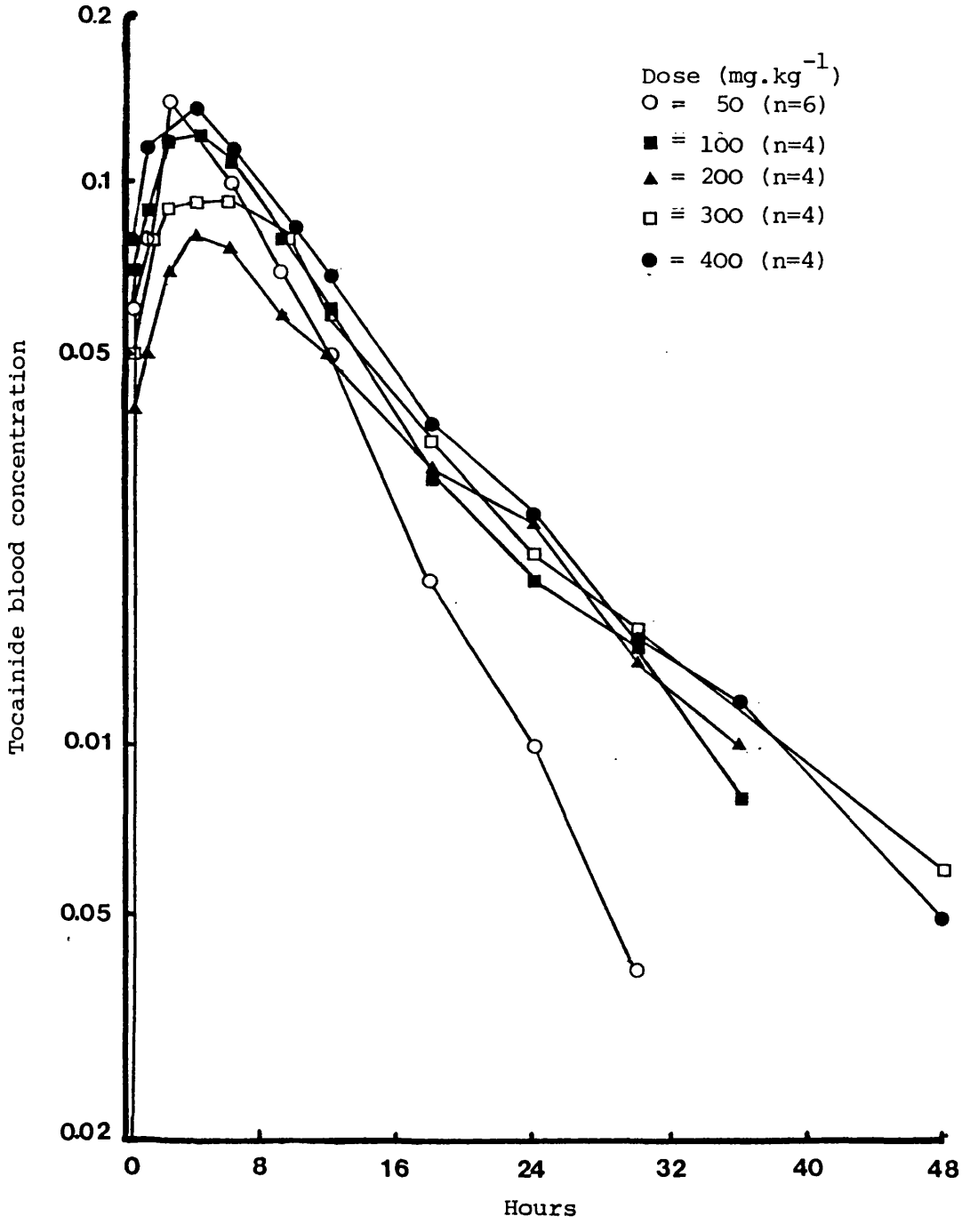



Figure 3.17 Superposition plot of the means of blood tocainide following four oral doses

Table 3.10 Systemic availability of tocainide following oral doses of 50, 100, 200, 300 and 400 $mg.kg.^{-1}$

| Oral Dose ($mg.kg^{-1}$) | Mean $AUC_{p.o.}$ ($\mu g.ml^{-1}.min$) | Normalized Mean $AUC_{i.v.}$ ($\mu g.ml^{-1}.min$) | Systemic availability |
|---|---|---|---|
| 50 | 4303.01 | 5803.46 | 0.7414 |
| 100 | 11043.49 | 11606.93 | 0.9514 |
| 200 | 18530.37 | 23213.85 | 0.7982 |
| 300 | 32675.03 | 34820.78 | 0.9384 |
| 400 | 50007.51 | 46427.71 | 1.0771 |
| | | | Mean±SEM 0.9412±0.0570 |

Systemic availability (F) of orally administered tocainide was calculated as the ratio of the AUC at each oral dose to the mean of all AUC's obtained after intravenous administration normalised for dose. A mean figure of (94.1 ± 5.7%) was obtained from the 100, 200, 300 and 400 $mg.kg^{-1}$ doses. The data for the 50 $mg.kg^{-1}$ dose were excluded from this analysis because $t_{\frac{1}{2}}$ was significantly shorter and the dose normalized blood concentration-time plot was not superimposable with those obtained from other doses. Since tocainide is completely absorped from the gut (3.6), not metabolised within the gut wall (3.5) and since renal clearance can be assumed to be equal for intravenous and for oral dosing a value for hepatic extraction (E) of tocainide and be obtained (E=1 -F) and is equal to 0.06.

Apart from slight sedation no other clinical signs of toxicity were evident in animals recieving 50, 100, 200 and 300 $mg.kg^{-1}$ orally. Four out of five rats in the 400 $mg.kg^{-1}$ dose group developed seizures within one hour of dosing, one rat died in the fourth hour after dosing and the rest survived.

3.5 Single intraperitoneal dose of tocainide

Tocainide in 100 $mg.ml^{-1}$ saline was given by intraperitoneal injection to six rats (body weight 455-500 g) in a dose of 50 $mg.kg^{-1}$. Blood samples were taken at appropriate time intervals before and after administration and were assayed for tocainide. The individual blood concentration-time data are given in Appendix 8 together with the means for the group. Mean blood concentration of tocainide ranged between 1.51 and 11.05 $\mu g.ml^{-1}$ and the maximum concentrations were reached between 1 and 2.5 hours. The mean blood concentration-time data are plotted in Figure 3.18 on a linear scale and in Figure 3.18 on a logarithmic scale with respect to time. The latter figure shows that the logarithm of the terminal phase of decline in drug concentration with time is linear. The kinetic parameters $k_d$, $t_{\frac{1}{2}}$ and $AUC_{o\rightarrow\alpha}$ were calculated for each animal as described in Appendix 20 and the mean data are given in Table 3.11. This table also shows kineitc parameters calculated for the same dose of tocainide administered to six rats by the oral route (3.4). The terminal blood $t_{\frac{1}{2}}$ of tocainide was less after intraperitoneal than after oral administration ($p<0.001$ by Student's 't' test) but $AUC_{o\rightarrow\alpha}$ showed no statistically significant differences when the two routes of administration were compared indicating that after oral administration of tocainide there is no loss of drug prior to its reaching the liver.

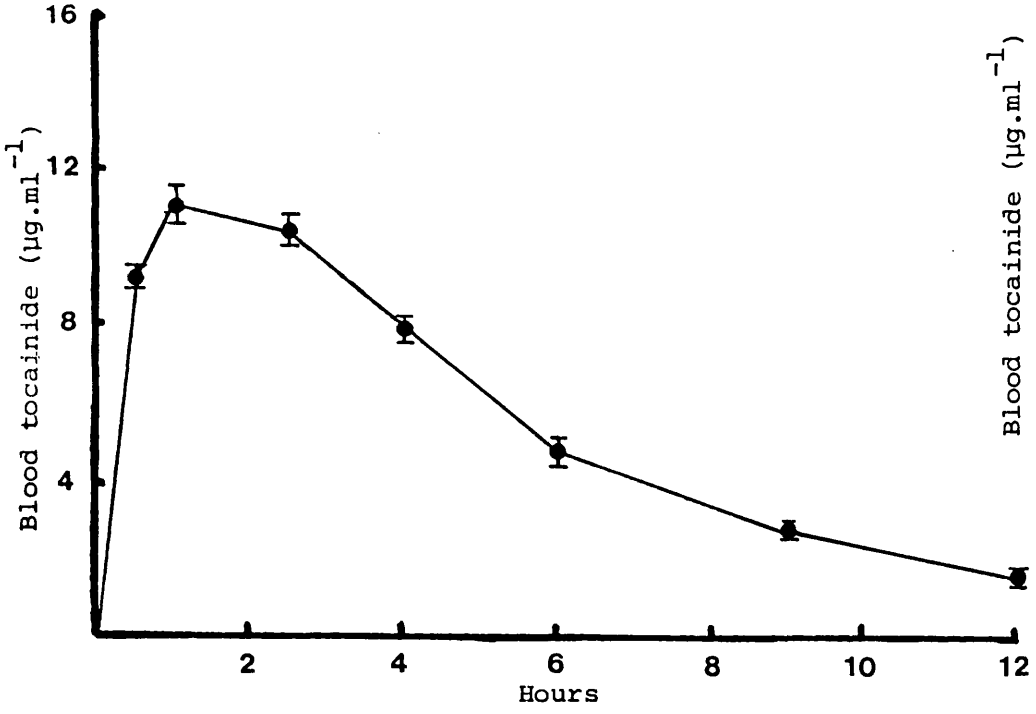



Figure 3.18 Mean (±S.E.M.) blood concentrations of tocainide following 50 mg.kg by intraperitoneal injection

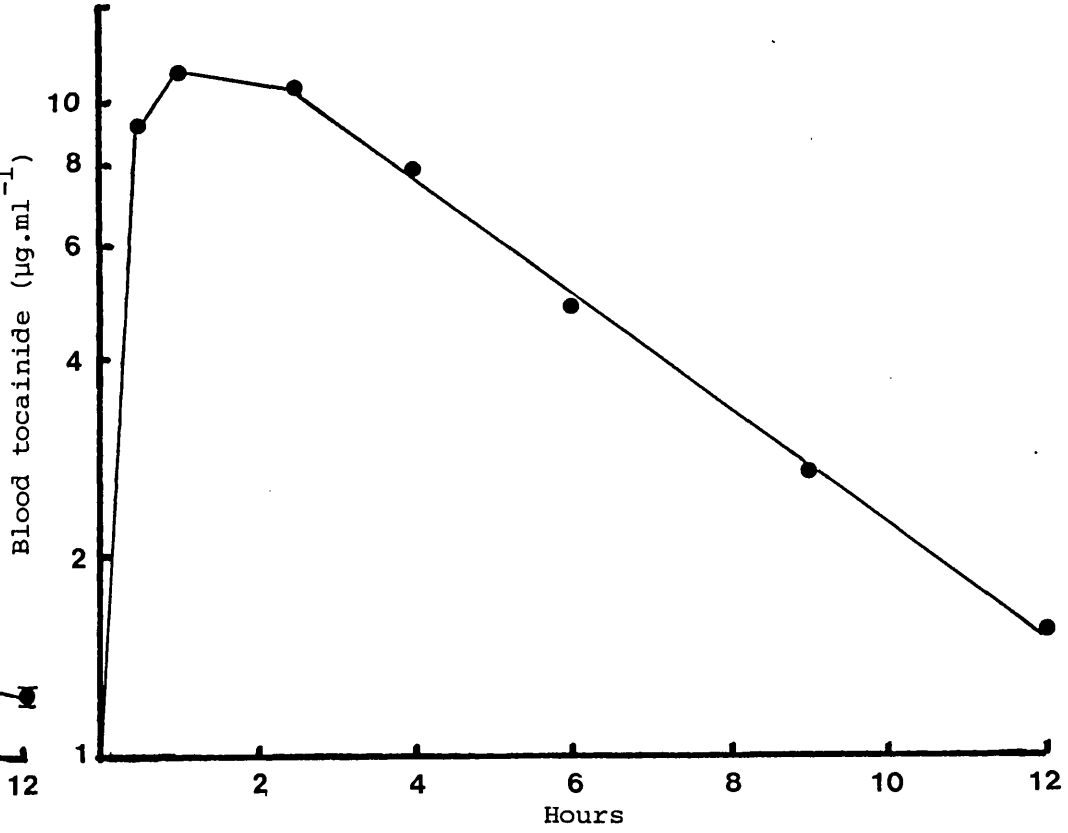



Figure 3.19 Semilogarithmic plots of mean blood concentrations of tocainide following 50 mg.kg$^{-1}$ by intraperitoneal injection.

Table 3.11 Comparison of the kinetic parameters of tocainide following i.p. and p.o. administration (50 $mg.kg^{-1}$) (Mean ± SEM)

| | Body weight (g) | n | $k_d$ ($min^{-1}$) | $t_{\frac{1}{2}}$ (min) | AUC ($\mu g.ml^{-1}.min$) |
|---|---|---|---|---|---|
| i.p. | 483.33 ±6.91 | 6 | 0.0034 ±0.0001 | 203.2** ±6.9 | 4489.08 ±203.21 |
| p.o. | 524.17 ±14.17 | 6 | 0.0026 ±0.0002 | 276.6 ±19.7 | 4303.01 ±544.32 |

** $p<0.001$

3.6 Completeness of absorption of orally administered tocainide

Tocainide was given by mouth to three rats (body weights 450, 460 and 465 g) in a dose of 200 $mg.kg^{-1}$. All faeces passed after dosing were collected and 48 hours after dosing the animals were killed by cervical dislocation. The entire alimentary tract of each animal was then removed and washed through with isotonic saline until the washings were clear. Faeces and washings from each animal were then combined and assayed for tocainide. The mean percentage recovery of unchanged drug was 0.025 ± 0.006% indicating essentially complete absorption of tocainide from the alimentary tract.

3.7 Multiple intravenous administration of tocainide

Tocainide was given by i.v. bolus injection to six rats (body weights 450-570 kg) in a dose of 20 $mg.kg^{-1}$ every 8 hours for seven doses. Blood samples were taken prior to and after the first dose, after the sixth dose and before and after the seventh doses, at appropriate time intervals. Because the total procedure involved more blood samples than were taken in the single i.v. dose study, the number of blood samples taken to describe the first and the seventh doses had to be reduced for reasons associated with the method of sampling (cutting off the tip of the rat tail). Consequently it was not possible to define the rapid ($\alpha$) phase of drug distribution adequately. The individual and the mean blood concentration-time data appear in Appendix 10.

Table 3.12 shows the individual and mean values for $k_d$, $t_{\frac{1}{2}}$ AUC, $Vd_{extrap}$ and total body clearance for the first and for the seventh doses of tocainide. This table also gives the mean blood concentration at steady state ($C_{\overline{ss}}$) which was calculated from the seventh dose by the relationship:

$$C_{\overline{ss}} = \frac{AUC_{o \to 8}}{T}$$

When the kinetic data for the first and for the seventh dose were compared no significant statistical differences were found by Student's 't' test and by the Wilcoxon signed rank test. The mean blood concentration-time curves are shown graphically in Figure 3.15. The difference in mean $t_{\frac{1}{2}}$ (145.8 min for the first dose and 203.8 min for the seventh dose) was almost entirely due to rat 3 in which the individual $t_{\frac{1}{2}}$ increased over threefold. It was also noted that this rat convulsed immediately after administration of the sixth and of

the seventh doses, the duration of convulsions being about 30 mins on each occasion. The other five rats exhibited less than normal activity after the sixth and seventh doses.

Thus in five of the six rats studied under the stated conditions of multiple i.v. dosing the kinetics of tocainide were apparently dose-independent.

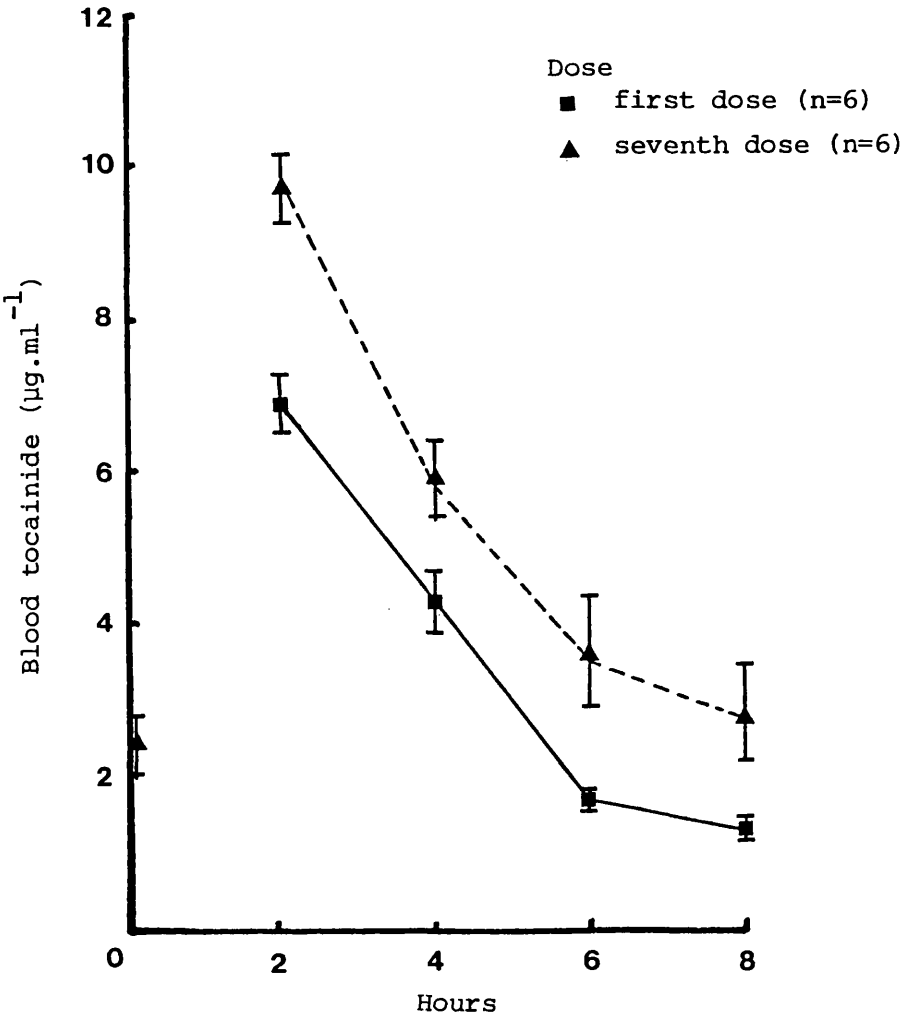



Figure 3.20 Means ± S.E.M. of blood tocainide concentration following the first and the seventh dose of tocainide 50 $mg.kg^{-1}$ intravenously every 8 hours.

Table 3.12 Pharmacokinetic parameters following tocainide 20 $mg.kg^{-1}$ by intravenous injection every 8 hours for seven doses

| Rat no | Weight (g) | After the First Dose | | | | | After the Seventh Dose | | | | | $C_{\overline{ss}}$** $\mu g.ml^{-1}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $k_d$ (min) | $t_{\frac{1}{2}}$ (min) | $AUC_{0\rightarrow\infty}$ $\mu g.ml^{-1}.min$ | $Vd_{extrap}$ $(l.kg^{-1})$ | Body Clearance $ml.min^{-1}.kg^{-1}$ | $k_d$ (min) | $t_{\frac{1}{2}}$ (min) | $AUC_{0\rightarrow8}$ | $Vd_{extrap}$ $(l.kg^{-1}$ | Body clearance $ml.min^{-1}.kg^{-1}$ | |
| 1 | 570 | 0.0039 | 177.7 | 2021.85 | 2.69 | 9.89 | 0.0048 | 148.3 | 1994.40 | 1.40 | 10.03 | 3.09 |
| 2 | 540 | 0.0044 | 156.6 | 2813.40 | 1.67 | 7.11 | 0.0032 | 217.0 | 2756.40 | 1.46 | 7.25 | 4.52 |
| 3 * | 500 | 0.0061 | 113.4 | 3057.60 | 1.12 | 6.54 | 0.0019 | 368.0 | 3550.40 | 1.45 | 5.63 | 5.36 |
| 4 | 450 | 0.0052 | 132.9 | 2895.44 | 1.38 | 12.69 | 0.0047 | 148.5 | 1959.00 | 1.40 | 10.21 | 4.90 |
| 5 | 460 | 0.0051 | 136.7 | 2478.02 | 1.66 | 8.07 | 0.0045 | 153.4 | 2509.80 | 1.20 | 7.97 | 4.15 |
| 6 | 450 | 0.0044 | 157.4 | 2835.35 | 1.70 | 7.21 | 0.0037 | 187.8 | 2658.60 | 1.39 | 7.69 | 4.56 |
| Mean ±SEM | 495 ±20.78 | 0.0048 ±0.0003 | 145.8 ± 9.2 | 2683.01 ±153.27 | 1.70 ± 0.22 | 8.58 ±0.95 | 0.0038 ±0.0004 | 203.8 ±34.4 | 2571.43 ±238.89 | 1.38 ±0.04 | 8.13 ±0.71 | 4.43 ±0.31 |

* No 3 convulsed for 30 min after the sixth and after the seventh dose

**$C_{\overline{ss}}$ mean concentration of drug in the blood at steady state

3.8 Multiple oral doses of tocainide

Tocainide was given by mouth to five rats (body weights 470-495 g) in a dose of 200 $mg.kg^{-1}$ every 12 hours for ten doses. Blood samples were taken at appropriate time intervals before and after the first dose, at six and nine hours after the first and fifth doses and before and after the tenth dose. The individual blood concentration-time data and the mean data for the group are given in Appendix 11. Table 3.12 shows the individual and mean values for $k_d$, $t_{\frac{1}{2}}$, AUC and total body clearance for the first and for the seventh dose of tocainide. The table also gives the mean blood concentration of tocainide at steady state ($C_{\overline{ss}}$) which was calculated from the tenth dose by the relationship:

$$C_{\overline{ss}} = \frac{AUC_{0 \to 12\ hr}}{T}$$

where T is the dose interval.

The mean blood concentration-time curves are also shown on a linear scale in Figure 3.21 and on a logarithm scale in Figure 3.22. The limitations imposed by the necessarily reduced freqency of blood sampling in this experiment resulted in there being fewer concentration points on which to base the estimation of $t_{\frac{1}{2}}$. For the first dose $t_{\frac{1}{2}}$ was based on the 6, 9 and 12 hour samples and for the tenth dose $t_{\frac{1}{2}}$ was estimated from samples obtained between one hour after the peak concentration and the 12 hour sample. In rats 3 and 5, $t_{\frac{1}{2}}$ increased substantially after the tenth dose and both these rats showed clinical signs of toxicity after the tenth dose had been given. No change in $t_{\frac{1}{2}}$ and no signs of toxicity were noted in the other animals. When the group means are compared no statistically significant difference were found in $t_{\frac{1}{2}}$ when the first and tenth doses are compared.

$AUC_{0\to\alpha}$ for the first dose was compared with $AUC_{0\to 12\ hr}$ (Wagner et al 1965 ) for the tenth dose and no significant difference was found for the group of rats as a whole, although it was noted that in rat 3 AUC had almost doubled after the tenth dose.

It is appreciated that the reduced frequency of blood sampling in this study has meant that the estimation of $t_{\frac{1}{2}}$ for the first dose and therefore $AUC_{0\to\alpha}$ is less secure than would ideally be the case. Nevertheless, it would appear that in the stated condition of multiple oral dosing in 3 of the 5 rats the kinetics of **tocaenide** were apparently dose independent.

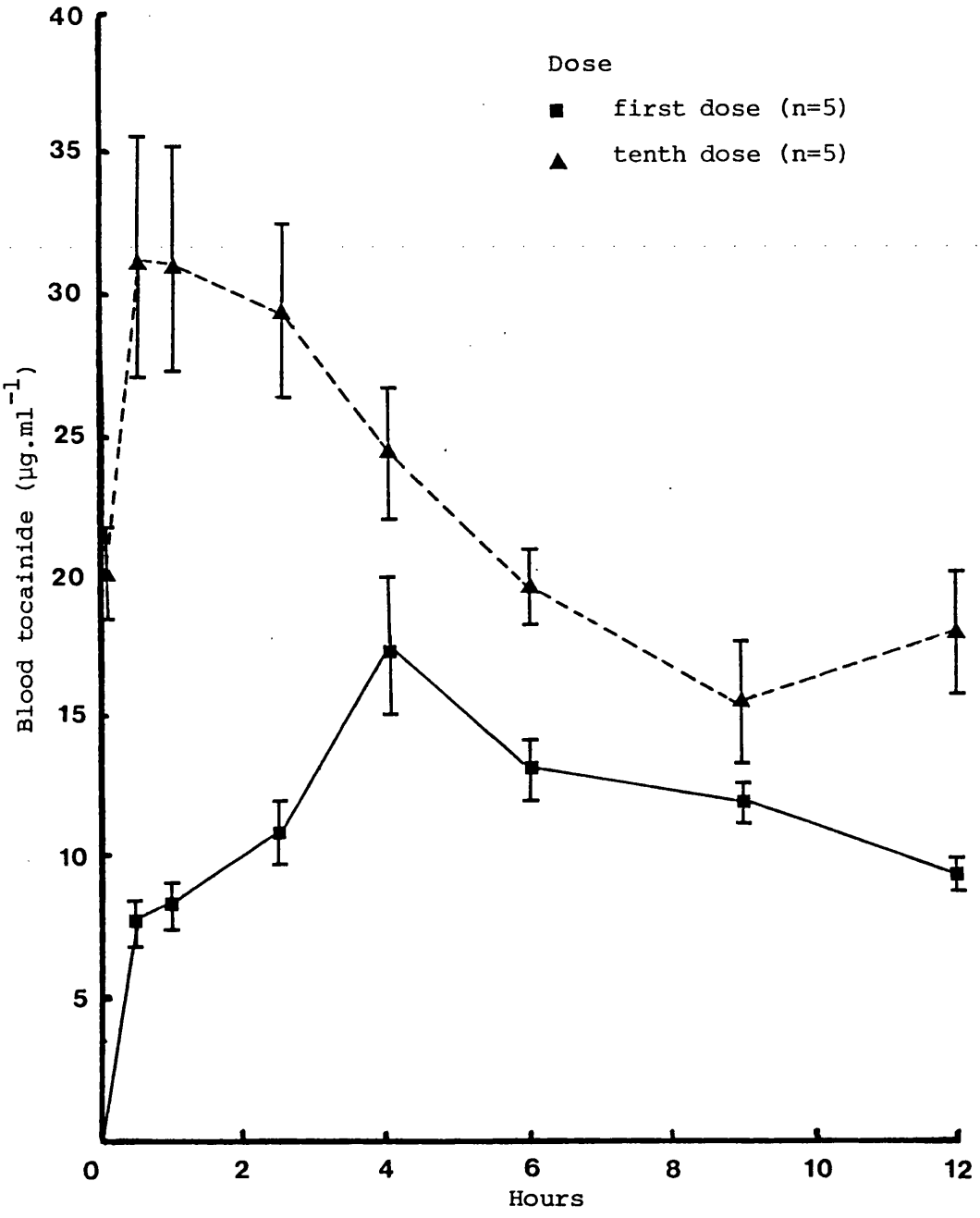



Figure 3.21 Mean ± S.E.M. of blood tocainide concentration following the first and the tenth dose of multiple oral administration of a 200 $mg.kg^{-1}$ dose every 12 hours

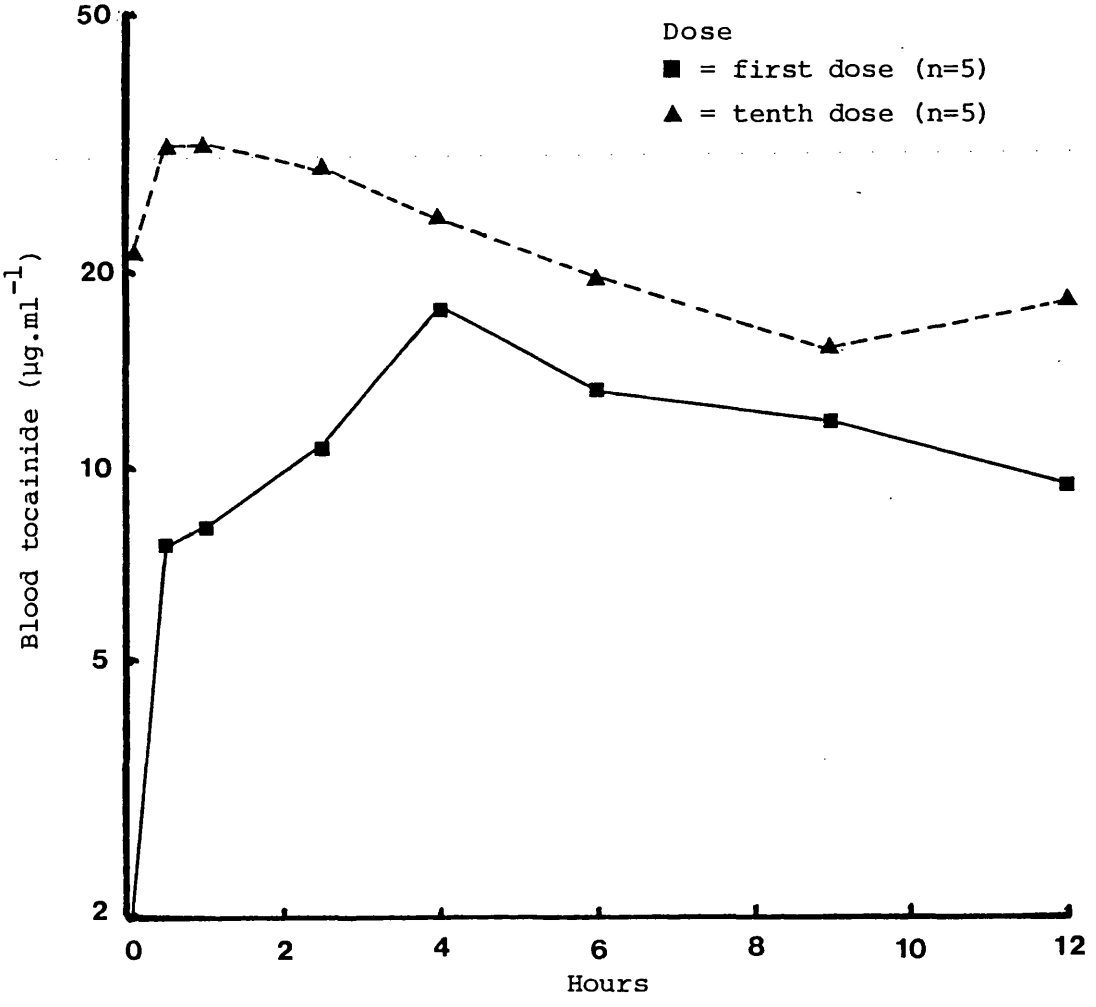



Figure 3.22 Mean of blood tocainide concentration following the first and the tenth dose of tocainide 200 $mg.kg^{-1}$ orally every 12 hours.

Table 3.13 Pharmacokinetic parameters following tocainide 200 mg.kg$^{-1}$ by mouth every 12 hours for ten doses

| Rat no | Weight (g) | After the first dose | | | | After the tenth dose | | | | $C_{\overline{ss}}$ |
|---|---|---|---|---|---|---|---|---|---|---|
| | | $k_d$ (min$^{-1}$) | $t_{\frac{1}{2}}$ (hr) | $AUC_{0\rightarrow\infty}$ µg.ml$^{-1}$.min | Body Clearance ml.min$^{-1}$.kg$^{-1}$ | $k_d$ (min$^{-1}$) | $t_{\frac{1}{2}}$ (hr) | $AUC_{0\rightarrow 12hr}$ µg.ml$^{-1}$.min | Body Clearance ml.min$^{-1}$.kg$^{-1}$ | |
| 1 | 475 | 0.0010 | 11.2 | 20500.95 | 9.75 | 0.0016 | 7.3 | 18795.00 | 10.64 | 13.77 |
| 2 | 495 | 0.0014 | 8.4 | 14849.99 | 13.47 | 0.0013 | 8.7 | 13563.90 | 14.74 | 10.22 |
| 3 | 480 | 0.0007 | 15.8 | 10850.46 | 18.43 | 0.0004 | 31.1 | 19225.80 | 10.40 | 9.71 |
| 4 | 475 | 0.0011 | 10.1 | 16493.70 | 12.12 | 0.0006 | 18.4 | 12721.50 | 15.72 | 12.46 |
| 5 | 470 | 0.0010 | 11.2 | 20529.89 | 9.74 | 0.0013 | 9.0 | 14200.05 | 14.08 | 13.48 |
| Mean | 479 | 0.0010 | 11.34 | 16645.00 | 12.70 | 0.0010 | 14.9 | 15.701.25 | 13.2 | 11.99 |
| ±SEM | ±4.30 | ±0.0001 | ±1.23 | ±1827.30 | ±1.60 | ±0.0002 | ±4.50 | ±1372.83 | ±1.09 | ±0.76 |

# CHAPTER IV

## Effects of enzyme inducing agents on pharmacokinetics of lignocaine and tocainide in rats

## 4.1 Effects of 3,4 benzpyrene and of phenobarbitone on hepatic microsomal enzyme activity

Thirty-one rats, 300-350 g body weight, were given either sodium phenobarbitone (100 $mg.kg^{-1}$ in 1 $ml.kg^{-1}$ saline, daily for 4 days) by intraperitoneal injection or 3,4 benzpyrene (80 $mg.kg^{-1}$ in 10 $ml.kg^{-1}$ corn oil) by single intraperitoneal administration as described in Chapter 2. Thirty-two control rats received equivalents volumes of saline or of corn oil only by intraperitoneal injection. Twenty four hours after the last injection of phenobarbitone and 48 hours after 3,4-benzpyrene, the difference in hepatic enzyme activity between the two groups of rats was determined using the following indices of drug metabolism; (1) pentobarbitone sleeping time;

(2) liver weight;

(3) hepatic microsomal protein;

(4) cytochrome P-450 content

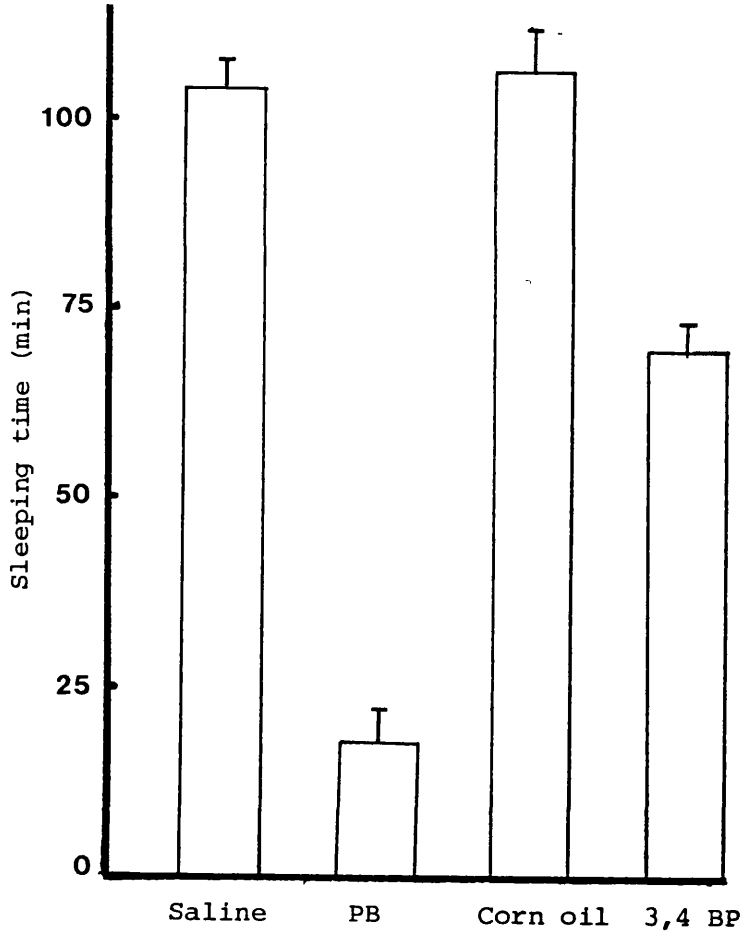
and (5) p-nitroanisole O-demethylase activity.

### 1. Pentobarbitone sleeping time

Groups of rats which had received either phenobarbitone, or 3,4-benzpyrene, or saline or corn oil as described above were given sodium pentobarbitone 40 $mg.kg^{-1}$ in a single intraperitoneal injection. After injection each animal was observed and tested for the presence of the righting reflex. Sleeping time was taken as the time from the loss of the righting reflex. The mean data are given in Figure 4.1. After phenobarbitone and after 3,4 benzpyrene, the mean pentobarbitone sleeping times were decreased by 82.9% ($p<0.01$) and by 35.0% ($p<0.01$) respectively.

Figure 4.1 Effects of phenobarbitone (PB) and of 3,4 benzpyrene (3,4 BP) on pentobarbitone sleeping time in rats (Mean±S.E.M.)

| | Saline | Phenobarbitone | Corn Oil | 3,4-benzpyrene |
|---|---|---|---|---|
| Sleeping time (min) | 104.2±3.5 (n=6) | 17.8±4.3 (n=5) | 106.2±5.5 (n=4) | 69.1±3.5 (n=5) |



2. Liver weight

Groups of rats which had received either phenobarbitone, or 3,4-benzpyrene or saline or corn oil as described above were killed by cervical dislocation. Livers were immediately removed and weighed. The mean values are shown in Table 4.1. There was no significant difference in liver weight between the two control groups but in the group which received phenobarbitone liver weight was on average 26.0% greater than in the group which received saline. Liver weight in the group which received 3,4 benzpyrene was not significantly different from that in the group which received corn oil.

Table 4.1 Effects of phenobarbitone and of 3,4 benzpyrene on liver weight in rats. (Mean ± SEM)

| Treatment | No of rats | g. liver.100 g of body weight$^{-1}$ |
|---|---|---|
| Control | 10 | 3.48 ± 0.13 |
| Phenobarbitone | 7 | 4.39 ± 0.32* |
| Corn Oil | 4 | 3.43 ± 0.12 |
| 3,4 benzpyrene | 6 | 3.67 ± 0.27 |

* $p<0.002$

3. Hepatocyte mixed function oxidase activity

a) Stimulation of microsomal protein and cytochrome P-450 formation

Cytochrome P-450 is an important constituent of the mixed function oxygenase system in the liver, and an increase in the concentration of cytochrome P-450 is taken to indicate an increase in hepatic metabolising capacity, i.e. enzyme induction.

Groups of rats received either phenobarbitone (100 $mg.kg^{-1}$, i.p., daily for 4 days) (4 rats) or 3,4 benzpyrene (80 $mg.kg^{-1}$, i.p. on one occassion) ( 4 rats) as described in Chapter 2. Twenty four hours after phenobarbitone and 48 hours after 3,4 benzpyrene the rats were killed and microsomes were prepared from each liver and assayed for protein concentration and cytochrome P-450 content according to the methods of Lowry (1951) and Omura and Sato (1964) respectively.

The mean microsomal protein concentration and the mean cytochrome P-450 content in the above groups of rats are present in Table 4.2. A significant increase in liver microsomal protein and in cytochrome P-450 content was found in phenobarbitone and in 3,4 benzpyrene treated rats when compared with saline and corn oil respectively. The results in both treated groups were similar and no significant difference between these groups was noted when the results are expressed as "per g liver". However, in the phenobarbitone animals there was a significant increase in liver weight which was not observed for the 3,4 benzpyrene rats. Therefore, the total increase in microsomal protein and in cytochrome P-450 content was greater after administration of phenobarbitone.

b) Stimulation of p-nitroanisole-O-demethylase

Change in the activity of microsomal O-demethylation of p-nitroanisole was used as an indication of alteration in overall liver microsomal drug metabolising ability, although this particular enzyme plays no part in the metabolism of lignocaine or of tocainide. Specific activity was observed by determining the amount of formaldehyde formed during incubation of substrate (p-nitroanisole) at different concentrations with hepatic microsomes from sixteen rats pretreated with saline (1 $ml.kg^{-1}$), phenobarbitone (100 $mg.kg^{-1}$, in saline, daily for

Table 4.2 Effect of phenobarbitone and of 3,4 benzpyrene on hepatic microsomal protein and on cytochrome P-450 content in rat. (Mean ± SEM)

| Treatment | n | Microsomal protein (mg.g liver$^{-1}$) | Cytochrome P-450 content (n mole.mg microsomal protein$^{-1}$) |
|---|---|---|---|
| Saline (1 ml.kg$^{-1}$, i.p. daily for 4 days) | 4 | 19.58±0.43 | 0.84±0.12 |
| Phenobarbitone (100mg.kg$^{-1}$,i.p. daily for 4 days in saline) | 4 | 23.00±1.29* | 1.57±0.21* |
| Corn oil (10 mg.kg$^{-1}$,i.p.) | 4 | 19.52±0.77 | 0.75±0.08 |
| 3,4 benzpyrene (80 mg.kg$^{-1}$,i.p. in corn oil | 4 | 22.58±0.99* | 1.46±0.10** |

* $p<0.05$

** $p<0.002$

4 days), corn oil (10 ml.kg$^{-1}$) or 3,4 benzpyrene (80 mg.kg$^{-1}$ in corn oil) i.e. four groups with four rats in each group. The method used is described in Chapter 2. The mean data for each group appear in Table 4.3. This shows that p-nitroanisole-O-demethylase activity increased in the phenobarbitone group almost three-fold and in the 3,4 benzpyrene group almost two and a half-fold when compared with the controls.

The kinetic behavior of normal and of stimulated enzymes was studied by determining the Michaelis-Menten constant ($K_m$) of p-nitroanisole. The data, plotted according to the method of Lineweaver and Burk (1934) were analysed statistically by least square linear regression in order to facilitate drawing the line of best fit. The results are shown in Figure 4.2. Phenobarbitone and 3,4 benzpyrene treatment resulted in an increased maximum velocity ($V_{max}$) of O-demethylase, whereas $K_m$ was unchanged, showing that an increase in enzyme activity was obtained without alteration in substrate affinity.

## 4.2 Effects of phenobarbitone, 3,4 benzpyrene, lignocaine and tocainide on haemodynamic parameters in the rat

In this section the effects of the individual drugs used in this chapter on various haemodynamic parameters are reported.

Rats received either phenobarbitone (100 mg.kg$^{-1}$, i.p. in 1 ml.kg$^{-1}$ saline, daily for 4 days) or 3,4 benzpyrene (80 mg.kg$^{-1}$ in 10 ml.kg$^{-1}$, corn oil, i.p., as a single dose) and were studied 24 hours or 48 hours after the last dose of phenobarbitone or 3,4 benzpyrene respectively. Other rats received lignocaine 5 mg.kg$^{-1}$ in saline or tocainide 35 mg.kg$^{-1}$ in saline as an i.v. bolus and were studied 30 minutes after injection of either lignocaine or tocainide. Control rats received either saline (1 ml.kg$^{-1}$, daily for 4 days) or corn oil (10 ml.kg$^{-1}$)

Table 4.3 Effect of phenobarbitone and of 3,4 benzpyrene on p-nitroanisole O-demethylase activity

(Mean ± SEM)

| | n | Formaldehyde formed ($\mu$moles.$hr^{-1}$.mg.$prol^{I-1}$) | $K_m$ (Mx10-4) | $V_{max}$ (m$\mu$ moles.mg.$prol^{-1}$.$min^{-1}$) |
|---|---|---|---|---|
| Saline (1 ml.$kg^{-1}$,i.p.,daily for 4 days) | 4 | 0.241±0.019 | 2.93±0.24 (n=2) | 12.27±0.77 (n=2) |
| Phenobarbitone (100.mg.$kg^{-1}$,i.p.,daily for 4 days, in saline) | 4 | 0.643±0.039* | 2.68±0.22 (n=3) | 46.48±3.41* (n=3) |
| Corn oil (10 ml.kg,i.p.) | 4 | 0.232±0.015 | 2.80±0.01 (n=2) | 12.00±0.73 (n=2) |
| 3,4 benzpyrene (80 mg.$kg^{-1}$,i.p.,in corn oil) | | 0.604±0.041* | 2.86±0.26 (n=3) | 35.50±5.11* (n=3) |

* $p<0.001$

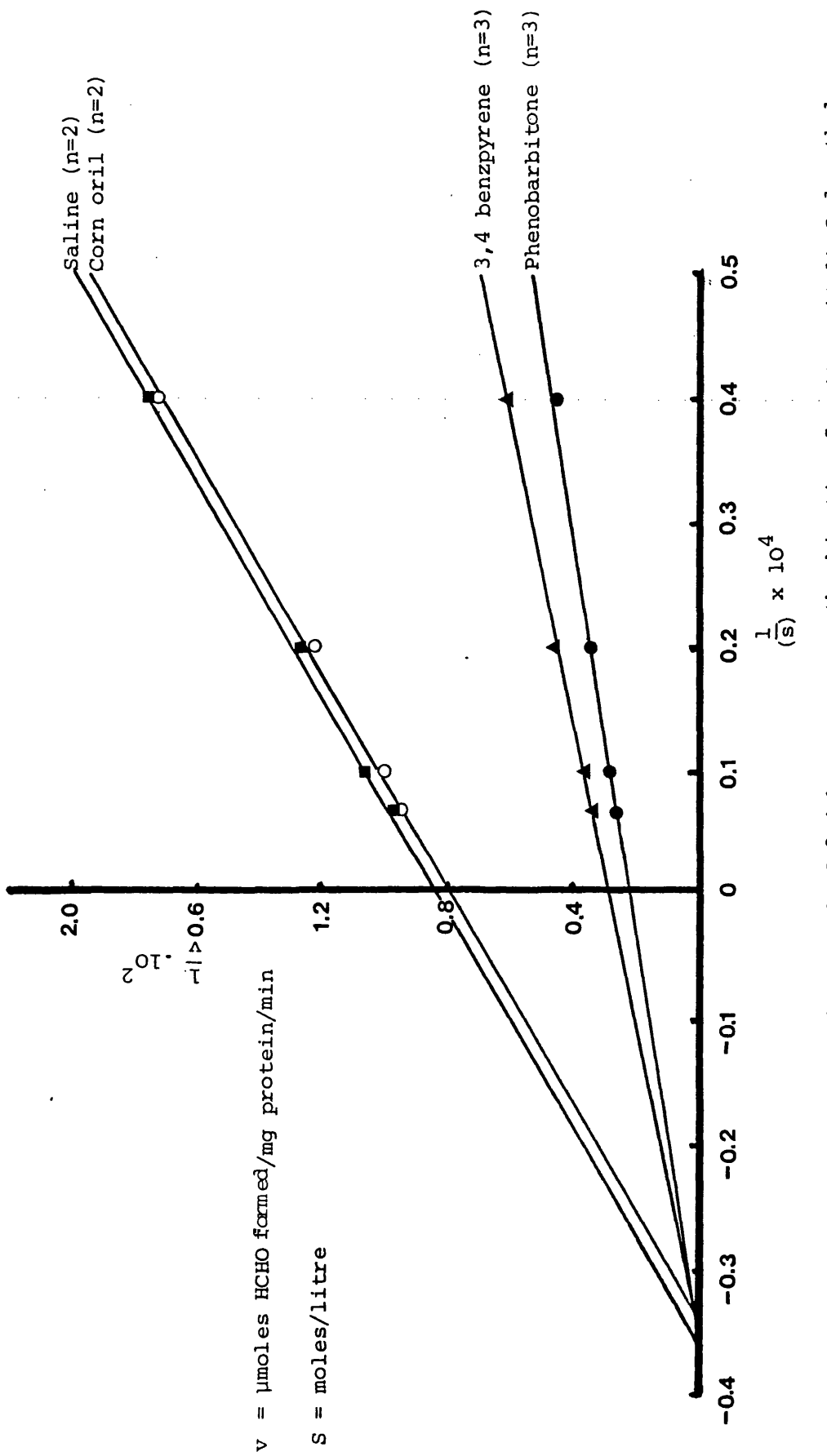



Figure 4.2 Effect of phenobarbitone and of 3,4 benzpyrene on the kinetic of p-nitroanisole-O-demethylase.

by intraperitoneal injection.. The timing of haemodynamic studies after drug administration was arranged to coincide as a) with known times of significant enzyme induction for phenobarbitone and 3,4 benzpyrene from section 4.1 and from previously published work (Ohnhaus et al, 1971; Nies et al, 1976) and b) in the case of lignocaine and tocainide with known times of high blood concentration when it was assumed that any haemodynamic effects would be maximal.

Mean arterial blood pressure, cardiac output and organ blood flow were measured as described in Chapter 2.

### 4.2.1 Effect on mean arterial pressure and on cardiac output

The data for arterial pressure and cardiac output for individual rats appear in Appendix 12-19 and the mean values are given in Table 4.4.

It is appreciated that a total analysis would have included the effect of combinations of the drugs as used in this work on these parameters but this was not undertaken. Rather the effect of only one combination namely phenobarbitone followed by tocainide (as in Chapter 4,5) was studied and the data for this are also given in Table 4.4.

Analysis of the data by student's 't' test and by the Wilcoxon signed rank test indicates that administration of phenobarbitone, lignocaine, tocainide or phenobarbitone followed by tocainide as stated caused no significant change in mean arterial pressure or in cardiac output when compared with the effect of administration of saline. By the same statistical tests, treatment with 3,4 benzpyrene producted no significant change in these parameters when compared with the group of rats which received corn oil.

Table 4.4 Influence of phenobarbitone, 3,4 benzpyrene, lignocaine, tocainide and phenobarbitone followed by tocainide on haemodynamic parameters in rat (Mean ± SEM)

| Treatment | n | mean arterial pressure (mm.Hg) | cardiac output ($ml.min^{-1}$) | cardiac output $ml.min^{-1}.kg^{-1}$) |
|---|---|---|---|---|
| saline | 10 | 116.5 ± 3.1 | 87.1 ± 9.3 | 192.4 ± 13.4 |
| phenobarbitone | 7 | 128.9 ± 3.0 | 107.5 ± 15.1 | 213.5 ± 25.6 |
| corn oil | 4 | 118.0 ± 3.2 | 94.5 ± 16.3 | 223.2 ± 32.2 |
| 3,4 benzpyrene | 6 | 113.3 ± 3.5 | 82.0 ± 3.9 | 188.4 ± 12.2 |
| lignocaine | 4 | 113.1 ± 4.0 | 121.7 ± 20.8 | 212.9 ± 15.1 |
| tocainide | 4 | 120.6 ± 6.9 | 109.1 ± 19.4 | 211.0 ± 29.0 |
| phenobarbitone + tocainide | 4 | 121.9 ± 5.2 | 78.7 ± 7.8 | 204.6 ± 11.0 |

4.2.2 Effect on liver blood flow and on liver weight

The data for liver blood flow and liver weight for indivdual animals are given in Appendix 12-19 and the mean values appear in Table 4.5. There were no significant differences by student's 't' test or by the Wilcoxon signed rank test in hepatosplanchnic flow (total liver blood flow) or liver weight between rats receiving saline and those receiving corn oil. Likewise, there were no significant differences in these paramenters when treatment with 3,4 benzpyrene was compared with treatment with corn oil alone or when treatment with lignocaine or tocainide was compared with treatment with saline alone.

Treatment with phenobarbitone however, was associated with a 39% increase in hepatosplanchnic flow (total liver blood flow) per 100 g body weight and a 26% increase in liver weight per 100 g body weight. The increase in hepatosplanchnic flow was accounted for by a significant

Table 4.5 Effects of phenobarbitone, 3,4 benzpyrene, lignocaine, tocainide and phenobarbitone followed by tocainide on liver weight and liver blood flow in rat

| | saline | phenobarbitone | corn oil | 3,4 benzpyrene | lignocaine | tocainide | phenobarbitone & tocainide |
|---|---|---|---|---|---|---|---|
| No of rats | 10 | 7 | 4 | 6 | 4 | 4 | 4 |
| Liver weight ($g.100g,bw^{-1}$) | 3.48±0.13 | 4.39±0.32*** | 3.43±0.12 | 3.67±0.27 | 3.55±0.24 | 2.74±0.37 | 4.43±0.11*** |
| GI tract and pancreas ($g.100g,bw^{-1}$) | 2.99±0.24 | 2.92±0.26 | 3.15±0.05 | 3.01±0.25 | 2.63±0.30 | 3.11±0.30 | 4.57±0.62 |
| Spleen weight ($g.100g,bw^{-1}$) | 0.20±0.01 | 0.17±0.01 | 0.18±0.01 | 0.16±0.01 | 0.19±0.01 | 0.15±0.02 | 0.20±0.04 |
| Hepatosplanchnic (liver) blood flow | | | | | | | |
| $ml.min^{-1}.100g,bw^{-1}$ | 4.06±0.43 | 5.67±0.56** | 4.00±0.37 | 4.16±0.34 | 4.02±0.44 | 4.06±0.22 | 5.85±0.65** |
| $ml.min^{-1}.g\ liver^{-1}$ | 1.17±0.11 | 1.34±0.17 | 1.17±0.12 | 1.15±0.09 | 1.18±0.13 | 1.20±0.09 | 1.32±0.14 |
| GI tract and pancreas blood flow[a] | | | | | | | |
| $ml.min^{-1}.100\ g,bw^{-1}$ | 2.70±0.30 | 3.69±0.21* | 2.62±0.18 | 2.83±0.37 | 2.88±0.41 | 3.14±0.23 | 4.48±0.58** |
| $ml.min^{-1}g\ liver^{-1}$ | 1.05±0.15 | 1.37±0.14 | 0.89±0.10 | 0.94±0.10 | 1.11±0.19 | 1.08±0.24 | 0.96±0.09 |
| Hepatic arterial blood flow[b] | | | | | | | |
| $ml.min^{-1}.100g,bw^{-1}$ | 1.04±0.20 | 1.62±0.51 | 0.96±0.11 | 0.96±0.14 | 0.74±0.03 | 0.57±0.10 | 1.02±0.10 |
| $ml.min^{-1}.g\ liver^{-1}$ | 0.30±0.05 | 0.39±0.12 | 0.28±0.03 | 0.28±0.05 | 0.21±0.006 | 0.17±0.03 | 0.23±0.02 |
| Spleen, blood flow[c] | | | | | | | |
| $ml.min^{-1}.100g,bw^{-1}$ | 0.32±0.03 | 0.36±0.05 | 0.46±0.09 | 0.36±0.05 | 0.40±0.04 | 0.36±0.06 | 0.36±0.06 |
| $ml.min^{-1}.gliver^{-1}$ | 1.87±0.25 | 2.09±0.26 | 2.44±0.53 | 2.26±0.24 | 2.14±0.43 | 2.44±0.38 | 1.76±0.10 |

a + c = portal flow;
b = hepatic artery flow;
a+b+c = hepatosplanchnic flow

* $p<0.05$ ** $p<0.02$ *** $p<0.002$

increase ($p<0.05$) in portal venous flow, that is, flow from the gastrointestinal (GI) tract and pancreas. Splenic blood flow and hepatic arterial flow to the liver did not increase significantly after phenobarbitone. Neither spleen weight nor the combined weight of the GI tract and pancreas were found to be affected by phenobarbitone. Since both hepatosplanchnic flow and liver weight were increased by phenobarbitone, liver blood flow when expressed as "per g of liver" was unchanged by phenobarbitone.

Administration of phenobarbitone followed by tocainide was associated with similar changes in liver weight and hepatosplanchnic flow to those found with phenobarbitone alone. The data are represented graphically in Figure 4.4

### 4.2.3 Effects on blood flow to other organs and on regional distribution of cardiac output

The data for blood flow to and weight of heart, lungs and kidneys for individual animals appear in Appendix 12-19 and the mean values are given in Table 4.6. No significant differences were found after any drug treatment. The individual values for the percentage of cardiac output received by the major organs are given in Appendix 12-19 and the mean values appear in Table 4.7. A significant increase in distribution to GI tract and pancreas was observed after treatment with phenobarbitone followed by tocainide ($p<0.05$) but not by treatment with phenobarbitone alone. Other treatments did not produce significant changes in the distribution of cardiac output.

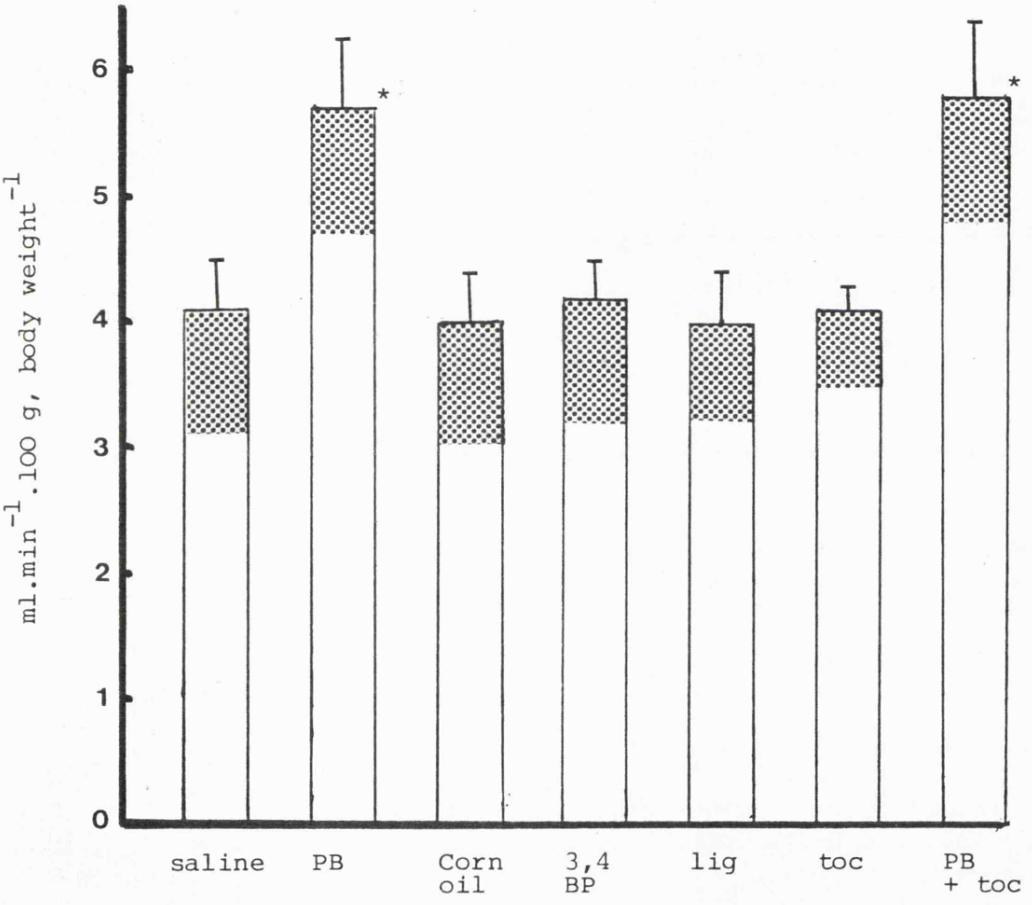



Figure 4.4 Effects of phenobarbitone (PB), 3,4 benzpyrene (3,4 BP), lignocaine, tocainide, and phenobarbitone followed by tocainide on liver blood flow (ml.min$^{-1}$.100 g, bw$^{-1}$) in rat. Hepatic arterial flow is represented by cross-hatched bars and portal venous flow by the unhatched bars. * $p<0.02$

Table 4.6 Effects of phenobarbitone, 3,4 benzpyrene, lignocaine, tocainide and phenobarbitone followed by tocainide on blood flow to major organs in rat

| | saline | phenobarbitone | corn oil | 3,4 benzpyrene | lignocaine | tocainide | phenobarbitone & tocainide |
|---|---|---|---|---|---|---|---|
| No of Rats | 10 | 7 | 4 | 6 | 4 | 4 | 4 |
| heart | | | | | | | |
| weight (g) | 1.7 ±0.1 | 1.4 ±0.1[a] | 1.4 ±0.2 | 1.5 ±0.1 | 1.4 ±0.1 | 1.7 ±0.2 | 1.3 ±0.1 |
| blood flow | | | | | | | |
| $ml.min^{-1}.100g,bw^{-1}$) | 0.82±0.13 | 2.28±0.89[a] | 1.80±0.73 | 0.92±0.12 | 1.03±0.20 | 0.90±0.06 | 0.92±0.19 |
| $ml.min^{-1}g^{-1}$ | 2.2 ±0.5 | 4.5 ±0.7[a]* | 5.1 ±2.2 | 2.7 ±0.4 | 3.4 ±0.7 | 2.6 ±0.3 | 2.6 ±0.4 |
| lungs | | | | | | | |
| weight (g) | 1.9 ±0.1 | 2.3 ±0.2 | 1.7 ±0.1 | 1.8 ±0.1 | 2.0 ±0.2 | 2.3 ±0.4 | 2.3 ±0.3 |
| blood flow | | | | | | | |
| $ml.min^{-1}.100g,bw^{-1}$) | 0.26±0.05 | 0.52±0.23 | 0.29±0.03 | 0.45±0.21 | 0.47±0.09 | 0.27±0.06 | 0.63±0.28 |
| $ml.min^{-1}g^{-1}$ | 0.7 ±0.1 | 1.2 ±0.6 | 0.7 ±0.1 | 0.9 ±0.2 | 1.1 ±0.2 | 0.7 ±0.2 | 1.5 ±0.5 |
| kidneys | | | | | | | |
| weight (g) | 3.7 ±0.1 | 3.7 ±0.1 | 2.9 ±0.3 | 3.5 ±0.2 | 3.3 ±0.1 | 3.7 ±0.2 | 3.3 ±0.4 |
| blood flow | | | | | | | |
| $ml.min^{-1}.100g,bw^{-1}$) | 3.22±0.50 | 3.41±0.83 | 3.14±0.40 | 3.29±0.25 | 3.07±0.32 | 3.77±0.61 | 3.16±0.32 |
| $ml.min^{-1}g^{-1}$) | 4.3 ±0.7 | 4.4 ±0.5 | 4.2 ±0.7 | 4.1 ±0.4 | 4.2 ±0.3 | 5.2 ±0.9 | 3.8 ±0.5 |

a n = 5

* $p<0.05$

Table 4.7 Percentage of cardiac output received by major organs

| | Cardiac Output (%) | | | | | | |
|---|---|---|---|---|---|---|---|
| No of rats | saline | phenobarbitone | corn oil | 3,4 benzpyrene | lignocaine | tocainide | phenobarbitone & tocainide |
| No of rats | 10 | 7 | 4 | 6 | 4 | 4 | 4 |
| Hepatosplanchnic | 24.0±1.5 | 27.5±3.9 | 18.5±1.5 | 22.1±1.1 | 18.8±1.4 | 20.0±1.9 | 28.5±2.1 |
| GI tract & pancreas | 15.9±1.3 | 18.6±3.2 | 12.3±1.3 | 15.1±1.7 | 13.4±1.4 | 15.5±1.9 | 21.7±1.8* |
| Liver | 6.2±1.0 | 7.1±1.7 | 4.4±0.4 | 5.1±0.6 | 3.5±0.2 | 2.7±0.4 | 5.1±0.6 |
| Spleen | 1.9±0.1 | 1.7±0.3 | 1.8±0.2 | 1.9±0.2 | 1.9±0.1 | 1.7±0.2 | 1.7±0.2 |
| Lungs | 1.4±0.3 | 2.1±0.8 | 1.2±0.1 | 2.3±0.9 | 2.3±0.6 | 1.1±0.2 | 3.0±1.3 |
| Kidneys | 18.9±2.1 | 16.0±1.4 | 13.5±1.9 | 18.1±2.0 | 14.5±0.7 | 18.3±2.8 | 15.8±2.4 |
| Heart | 4.8±0.6 | 6.6±0.1[a] | 7.5±2.7 | 4.8±0.4 | 5.0±1.1 | 4.1±0.4 | 4.0±0.9 |

a n = 5

* $p<0.05$

4.3 Pharmacokinetics of lignocaine given in a single intravenous dose in 3,4 benzpyrene and in phenobarbitone pretreated rats.

3,4 benzpyrene (80 $mg.kg^{-1}$) was administered by a single i.p. injection to six rats weighing 435 to 480 g, and phenobarbitone (100 $mg.kg^{-1}$) was administered, i.p. daily for 4 days to nine rats weighing 375 to 425 g. Lignocaine (5.0 $mg.kg^{-1}$) was administered to all rats in each group by single i.v. injection via the tail vein 48 hours after 3,4 benzpyrene and 24 hours after the last dose of phenobarbitone. Blood samples were taken at intervals and assayed for lignocaine. The data for individual rats and also the group means appear in Appendix 2. The group of eight rats which also received lignocaine 5 $mg.kg^{-1}$ intravenously but without prior administration of 3,4 benzpyrene or phenobarbitone in the earlier dose-ranging study (Chapter 3.1) served as a control group in this study. Mean blood lignocaine concentrations in control and in enzyme induced rats have been plotted on a linear scale (Figure 4.5) and on a logarithmic scale (Figure 4.6) against time.

Kinetic parameters for each animal (except for one rat in the phenobarbitone treated group) were derived using the non-linear least squares regression analysis programme NONLIN as described in Appendix 21. The blood concentration data in one animal in the phenobarbitone group exhibited a mono-exponential decline with time but are included in the analysis where appropriate. The mean values appear in Table 4.8.

When the data for the inital distribution space, Vp, are compared by student's 't' test, there was no difference between control and treated groups and there was no significant difference between both treated groups. $Vd_{extrap}$, $Vd_{area}$ and $Vd_{ss}$ were all significantly increased in the phenobarbitone treated group as compared with controls but these parameters were not increased in rats treated with 3,4 benzpyrene. It is noted that in 3,4 benzpyrene group, $k_e$ is signifcantly

increased but no significant changes occurred in $k_{12}$ and $k_{21}$. There were no difference in terminal half-life ($t_{\frac{1}{2}\beta}$) of lignocaine between control and enzyme induced rats and between the two treated groups. In the phenobarbitone-treated group the AUC for lignocaine was significantly decreased but this was not so far the group treated with 3,4 benzpyrene. Consequently total systemic clearance of lignocaine was signficantly increased by phenobarbitone treatment but not by treatment with 3,4 benzpyrene.

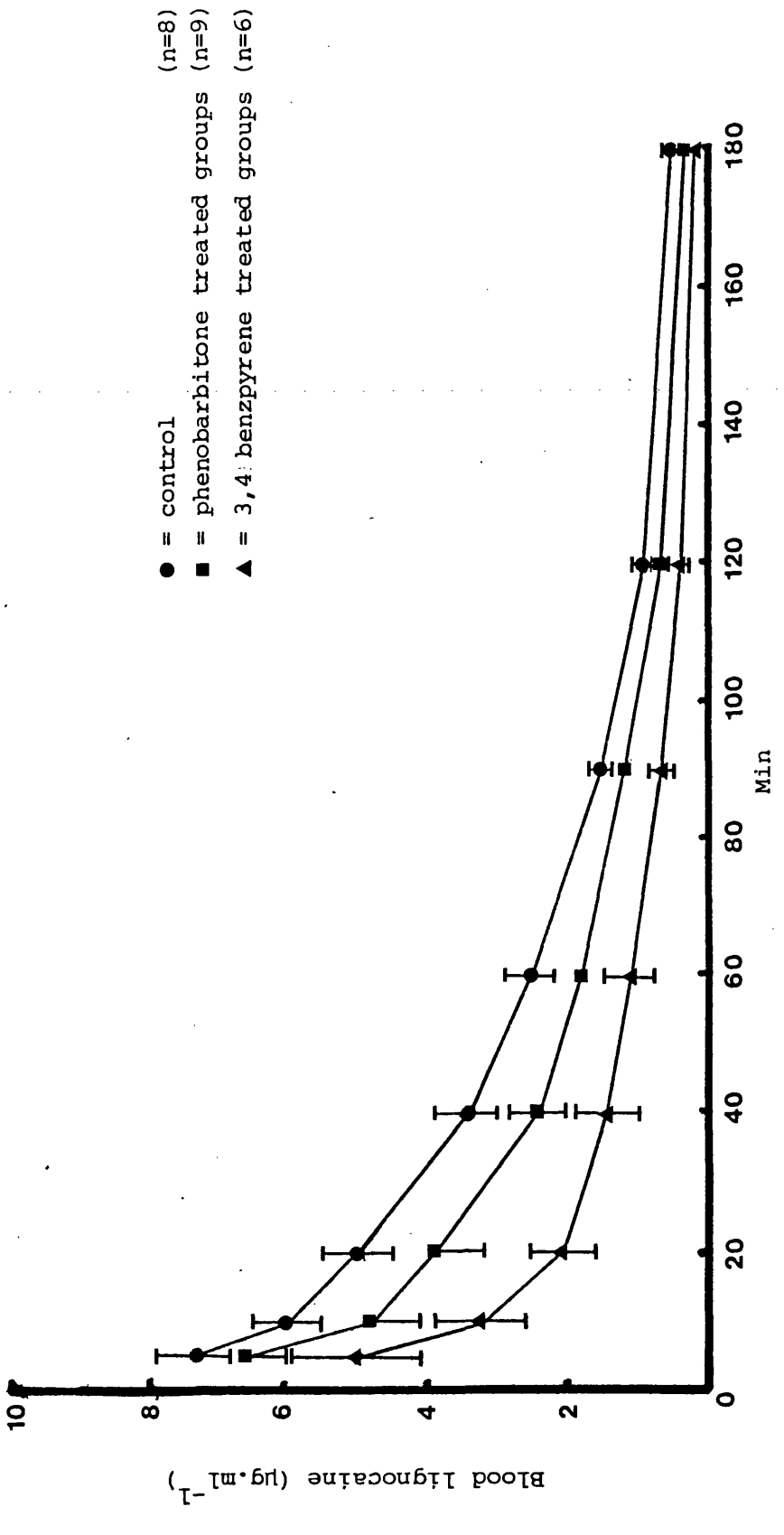



Figure 4.5 Blood concentration time graphs of lignocaine following 5mg/kg single intravenous injection in control, phenobarbitone and 3,4 benzpyrene treated rats. Mean ± S.E.M.

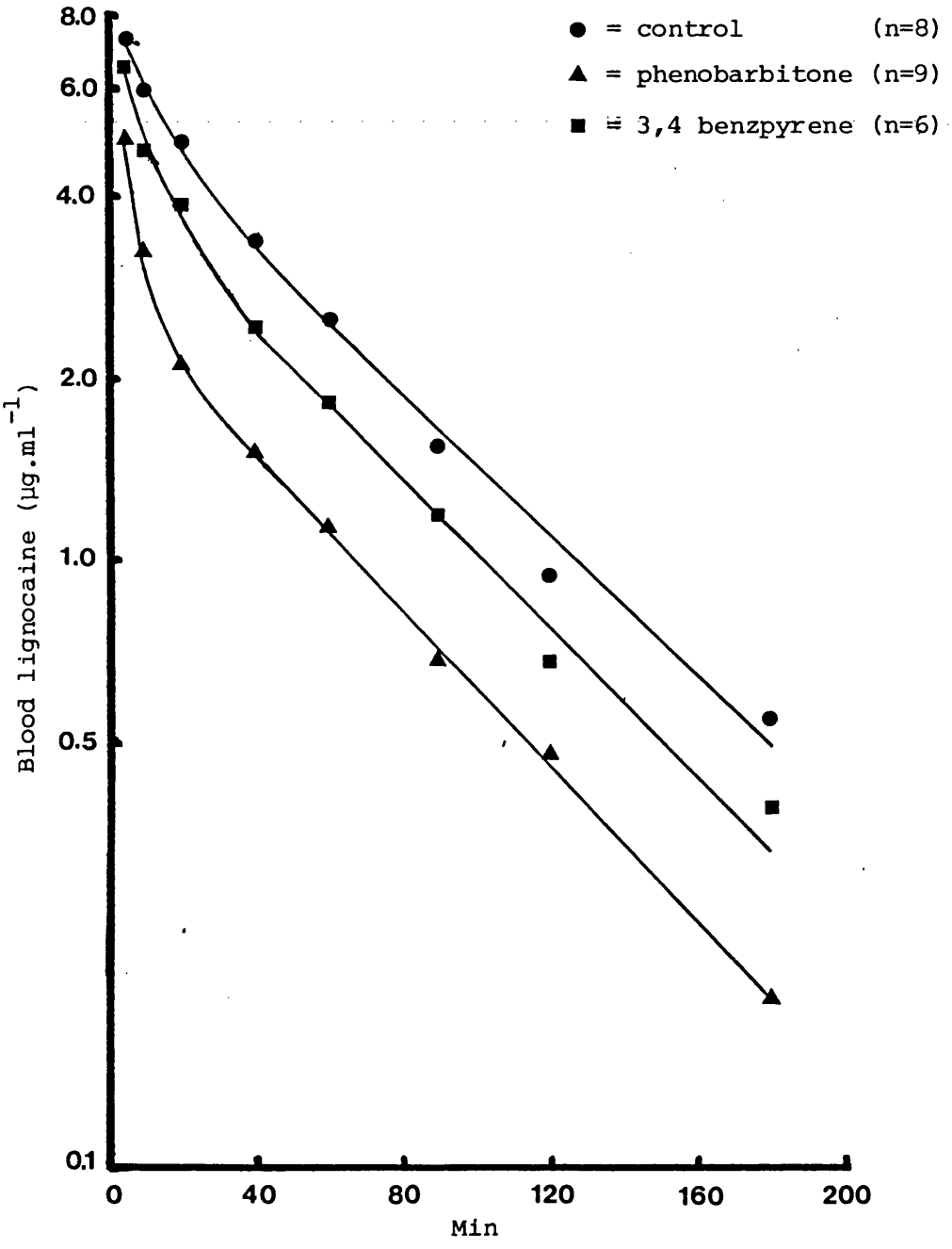



Figure 4.6 Mean blood concentration-time graphs of lignocaine following 5mg.kg$^{-1}$ intravenous injection in control, phenobarbitone and 3,4 benzpyrene treated rats.

Table 4.8 Mean kinetic data (±SEM) for lignocaine administered by a single intravenous dose (5 $mg.kg^{-1}$) in rats pretreated with 3,4 benzpyrene and with phenobarbitone

| Treatment | n | Body weight (g) | A ($\mu g.ml^{-1}$) | B ($\mu g.ml^{-1}$) | $\alpha$ ($min^{-1}$) | $\beta$ ($min^{-1}$) | $t_{\frac{1}{2}\alpha}$ (min) | $t_{\frac{1}{2}\beta}$ (min) |
|---|---|---|---|---|---|---|---|---|
| Control | 8 | 394 (±5.1) | 7.2[a] (±1.7) | 5.9 (±0.9) | 0.2208 (±0.0692) | 0.0148 (±0.0009) | 6.8 (±2.5) | 48.1 (±3.9) |
| 3,4 benzpyrene | 6 | 460 (±8.4) | 10.7 (±2.4) | 5.4 (±0.8) | 0.3463 (±0.0681) | 0.0196 (±0.0028) | 2.6 (±0.7) | 40.8 (±5.0) |
| Phenobarbitone | 9 | 396 (±8.6) | 6.8 (±1.4) | 2.5 (±0.6) | 0.1673 (±0.0305) | 0.0137 (±0.0014) | 5.8 (±1.7) | 58.2 (±10.2) |

| Treatment | n | Vp ($l.kg^{-1}$) | $Vd_{area}$ ($l.kg^{-1}$) | $Vd_{extrap}$ ($l.kg^{-1}$) | $Vd_{ss}$ ($l.kg^{-1}$) | $k_{12}$ ($min^{-1}$) | $k_{21}$ ($min^{-1}$) | $k_e$ ($min^{-1}$) | Total body clearance $ml.min^{-1}.100g,bw^{-1}$ | AUC ($\mu g.ml^{-1}.min$) |
|---|---|---|---|---|---|---|---|---|---|---|
| Control | 8 | 0.48 (±0.07) | 1.17 (±0.14) | 1.01 (±0.19) | 0.76 (±0.08) | 0.1119 (±0.0417) | 0.1101 (±0.0252) | 0.0275 (±0.0034) | 1.21 (±0.11) | 444.7 (±48.2) |
| 3,4 benzpyrene | 6 | 0.35 (±0.06) | 0.94 (±0.15) | 1.05 (±0.19) | 0.84 (±0.12) | 0.1912 (±0.0501) | 0.1227 (±0.0178) | 0.0511* (±0.0093) | 1.67 (±0.26) | 345.7 (±65.5) |
| Phenobarbitone | 9 | 0.74 (±0.18) | 2.61* (±0.58) | 3.13*** (±0.66) | 1.77** (±0.36) | 0.0847 (±0.0204) | 0.0548 (±0.0089) | 0.0408 (±0.0063) | 3.00** (±0.60) | 226.7** (±4.82) |

a n = 7

** $p<0.02$

* $p<0.05$

*** $p<0.001$

4.4 Pharmacokinetics of lignocaine given in a single oral dose in 3,4 benzpyrene and in phenobarbitone pretreated rats

3,4 benzpyrene (80 $mg.kg^{-1}$) was administered by a single i.p. injection to six rats weighing 430 to 475 g and phenobarbitone (100 $mg.kg^{-1}$) was administered i.p. daily for 4 days to six rats weighing 350 to 455 g. Lignocaine (70 $mg.kg^{-1}$) was administered to all rats in each group in a single oral dose 48 hours after 3,4 benzpyrene and 24 hours after the last dose of phenobarbitone. Blood samples were taken at intervals and assayed for lignocaine. The blood concentration time data for individual rats and the means for each group are presented in Appendix 4. The group of eight rats which also received lignocaine 70 $mg.kg^{-1}$ by single oral dose but without prior administration of 3,4 benzpyrene or phenobarbitone in the earlier dose-ranging study (Chapter 3.2) served as a control group in this study. The mean blood lignocaine concentrations in control and in enzymes induced rats have been plotted on a linear scale (Figure 4.7) and on a logarithmic scale (Figure 4.8) against time. The kinetic parameters for each group were derived as described in Appendix 20 and the mean data are listed in Table 4.9. This shows that there was no change in the rate constant for absorption ($k_a$) and for disposition ($k_d$) between control and enzyme-induced rats. Consequently blood $t_{\frac{1}{2}}$ of lignocaine was unchanged. However, the AUC after oral administration of lignocaine in both groups of enzyme-induced rats was singificantly less than in the control groups, the decrease in AUC being more marked in the phenobarbitone-treated group. Consequently apparent clearance of lignocaine by the oral route was significantly increased in the enzyme-induced rats, the increase being more marked in the phenobarbitone treated group. Change in $t_{\frac{1}{2}}$, AUC and in apparent oral clearance in control and in enzyme-induced rats are also shown in Figure 4.9.

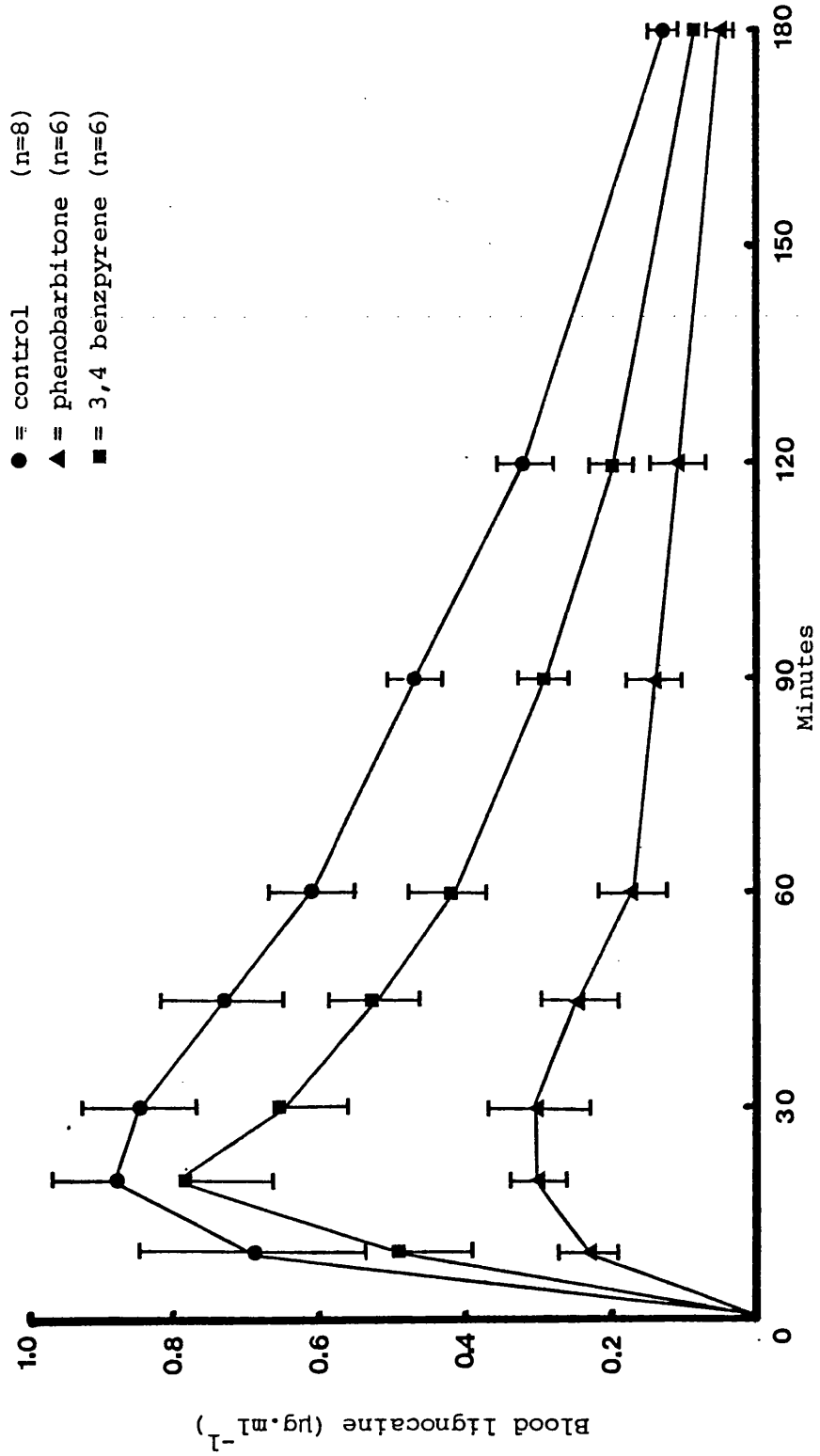



Figure 4.7 Blood concentration-time graphs of oral lignocaine (70 mg.kg) in control, phenobarbitone, 3,4 benzpyrene treated rats. (Mean ± S.E.M.)

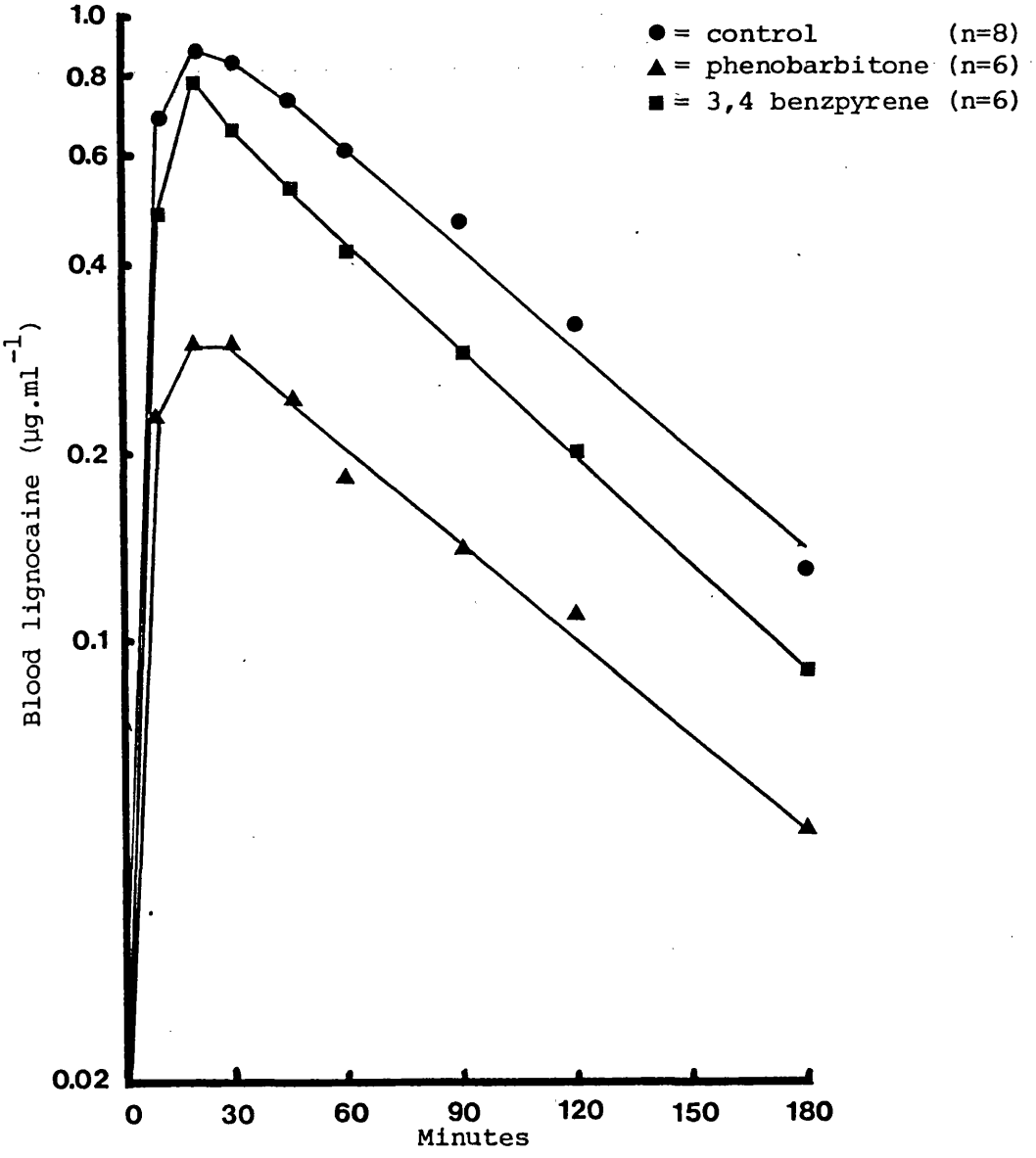



Figure 4.8 Mean blood concentration-time graphs of oral lignocaine (70 $mg.kg^{-1}$) in control, phenobarbitone and 3,4 benzpyrene treated rats

Table 4.9 Pharmacokinetic parameters of lignocaine following a single oral dose (70 $mg.kg^{-1}$) in control, 3,4 benzpyrene and phenobarbitone pretreated rats

| | Control | 3,4 benzpyrene | Phenobarbitone |
|---|---|---|---|
| n | 8 | 6 | 6 |
| Body weight (g) | 417.5 ±13.2 | 452.5 ±6.7 | 430.0 ±16.6 |
| $k_a$ ($min^{-1}$) | 0.1044 ±0.0174 | 0.1135 ±0.0269 | 0.0846 ±0.0200 |
| $k_d$ ($min^{-1}$) | 0.0121 ±0.0009 | 0.0129 ±0.0007 | 0.0116 ±0.0011 |
| $t_{\frac{1}{2}}$ (min) | 59.2 ±4.6 | 54.5 ±3.4 | 62.0 ±5.6 |
| $AUC_{po}$ ($\mu g.ml^{-1}.min$) | 103.10 ±9.48 | 65.74* ±7.63 | 33.14** ±7.94 |
| CL ($ml.min^{-1}.100g,bw^{-1}$) | 73.0 ±8.0 | 113.9* ±13.0 | 254.9** ±13.9 |

* $p<0.02$

** $p<0.001$

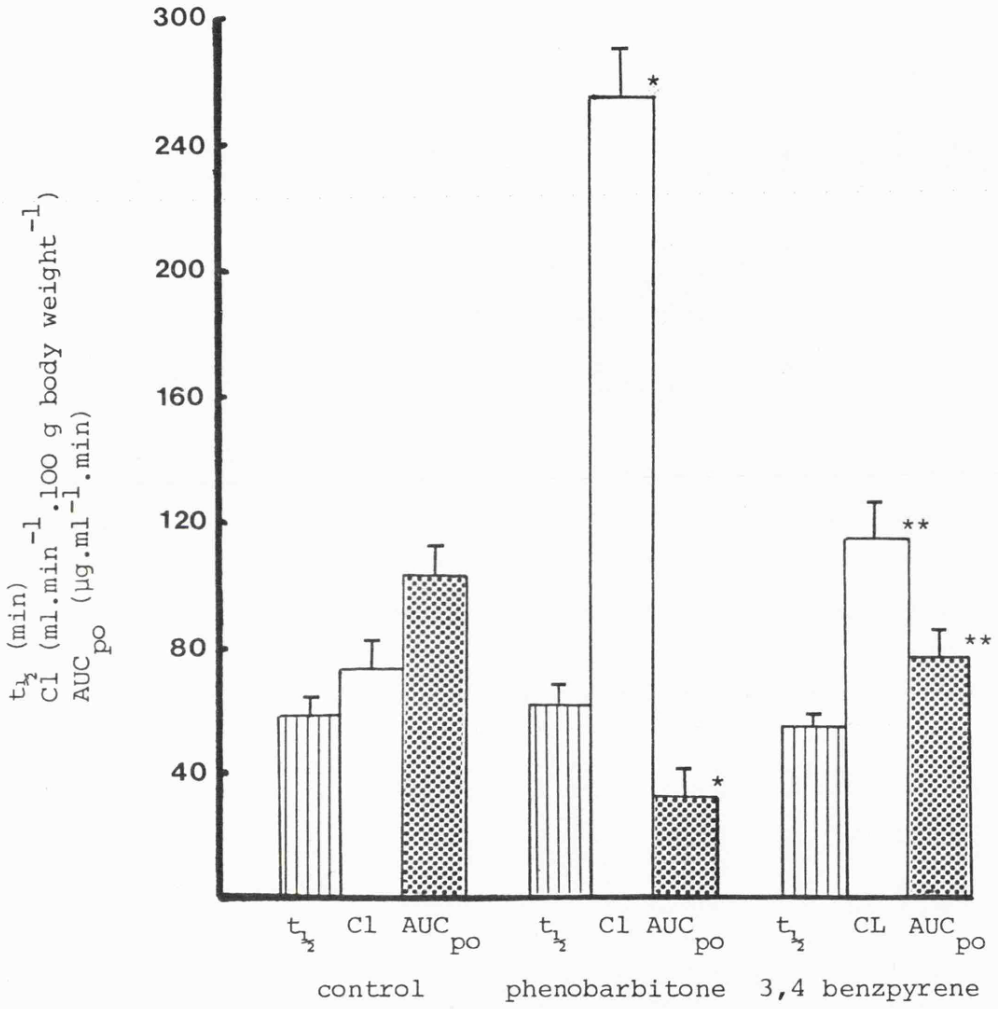



Figure 4.9 Effects of enzyme inducers on half-life, apparent oral clearance and area under blood concentration-time curves of lignocaine following 70 mg.kg$^{-1}$ by single oral administration

* $p<0.001$

** $p<0.02$

4.5 Pharmacokinetics of tocainide given in a single intravenous dose in 3,4 benzpyrene and in phenobarbitone pretreated rats

3,4 benzpyrene (80 $mg.kg^{-1}$) was administered by a single i.p. injection to six rats weighing 400 to 460 g and phenobarbitone (100 $mg.kg^{-1}$) was administered i.p. daily for 4 days to six rats weighing 470 to 525 g. Tocainide (35 $mg.kg^{-1}$) was administered to all rats in each group by a single i.v. infection via the tail vein 48 hours after 3,4 benzpyrene and 24 hours after the last dose of phenobarbitone. Blood samples were taken at intervals and assayed for tocainide. The data for individual rats and also the group means appear in Appendix 6. The group of five rats which also received tocainide 35 $mg.kg.^{-1}$ i.v. but without prior administration of 3,4-benzpyrene or phenobarbitone in the earlier dose-ranging study (Chapter 3.3) served as a control group for this study. Mean blood concentrations in control and in enzyme-induced rats have been plotted on a linear scale (Figure 4.10) and on a logarithmic scale (Figure 4.11) aginst time.

Kinetic parameters for each animal were derived using the nonlinear least squares regression analysis programme NONLIN as described in Appendix 21 and the mean values appear in Table 4.10.

When the data for the volume constants Vp, $Vd_{extrap}$, $Vd_{area}$ and $Vd_{ss}$ are compared, there was no significant difference between the control and the treated groups by student's 't' test. However, in both enzyme-induced groups of rats, there was a significant increase in the rate constant $k_e$ but no change was noted in the rate constants $k_{12}$ and $k_{21}$. There was no change in $t_{\frac{1}{2}\beta}$ in the group which received 3,4 benzpyrene but pretreatment with phenobarbitone was associated with an approximate halving of $t_{\frac{1}{2}\beta}$ ($p<0.02$) compared with the control group. AUC in both the enzyme-induced groups of rats was significantly

decreased as compared with controls, the decrease being greater after phenobarbitone treatment. Consequently total body clearance was increased in both enzyme-induced groups, the increase being greater in the group which received phenobarbitone.

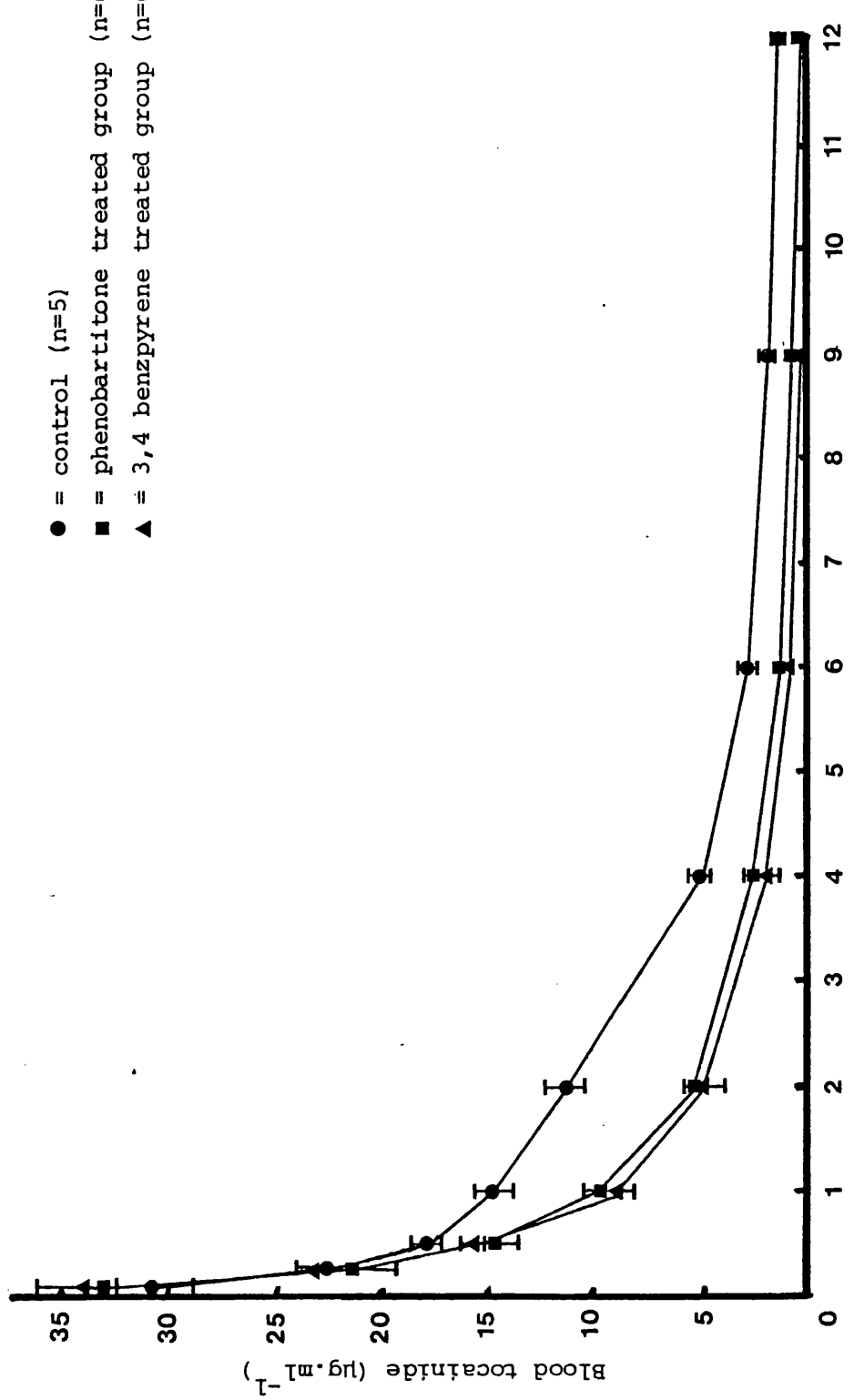



Figure 4.10 Blood concentration time graphs of tocainide following 35 mg.kg a single intravenous dose in control, phenobarbitone and 3,4 benzpyrene treated rats. (Mean ± S.E.M.)

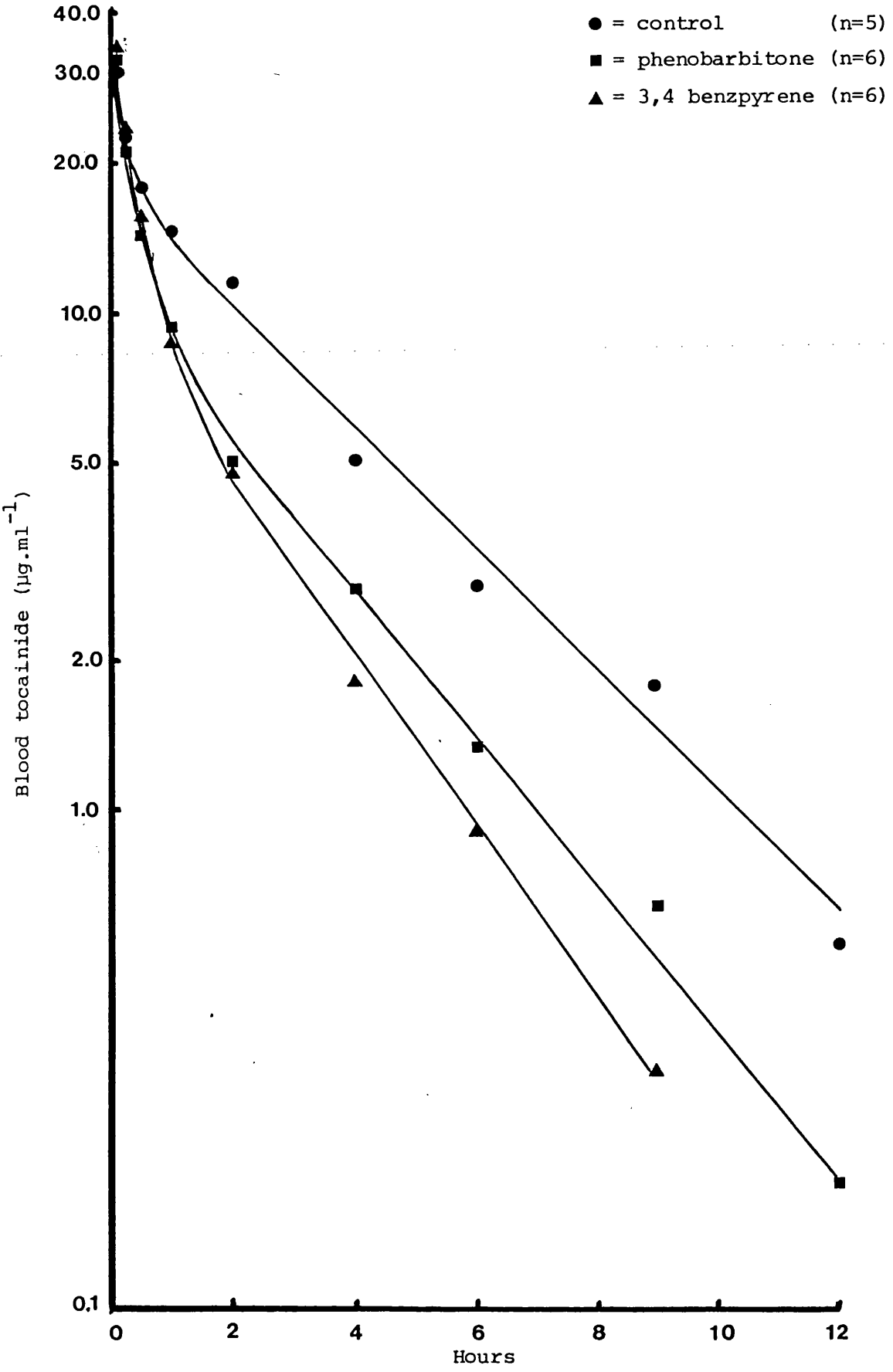



Figure 4.11 Mean blood concentration-time profiles of tocainide following 35 mg.kg single intravenous administration in control, phenobarbitone and 3,4 benzpyrene treated rats

Table 4.10 Mean kinetic data ±SEM for tocainide administered by single intravenous dose (35 mg.kg$^{-1}$) to rats pretreated with 3,4 benzpyrene and with phenobarbitone

| Treatment | n | Body weight | A (µg.ml$^{-1}$) | B (µg.ml$^{-1}$) | α (min$^{-1}$) | β (min$^{-1}$) | $t_{½}\alpha$ (min) | $t_{½}\beta$ (min) |
|---|---|---|---|---|---|---|---|---|
| Control | 5 | 518.0 (±15.9) | 23.5 (±9.6) | 20.8 (±0.9) | 0.1219 (±0.0325) | 0.0054 (±0.0002) | 7.0 (±1.3) | 127.9 (±4.9) |
| 3,4 benzpyrene | 6 | 421.7 (±9.8) | 31.8 (±4.0) | 12.5 (±1.8) | 0.0841 (±0.0181) | 0.0064 (±0.0007) | 10.4 (±2.2) | 115.6 (±14.4) |
| Phenobarbitone | 6 | 495.0 (±7.5) | 28.8 (±2.1) | 19.4 (±2.4) | 0.0846 (±0.0098) | 0.0125 (±0.0026) | 8.7 (±1.0) | 67.2*** (±12.0) |

| Treatment | n | Vp (l.kg$^{-1}$) | $Vd_{area}$ (l.kg$^{-1}$) | $Vd_{extrap}$ (l.kg$^{-1}$) | $Vd_{ss}$ (l.kg$^{-1}$) | $k_{12}$ (min$^{-1}$) | $k_{21}$ (min$^{-1}$) | $k_e$ (min$^{-1}$) | Total body clearance (ml.min$^{-1}$. 100 g,bw$^{-1}$) | AUC (µg.ml.$^{-1}$. min) |
|---|---|---|---|---|---|---|---|---|---|---|
| Control | 5 | 0.89 (±0.13) | 1.62 (±0.06) | 1.70 (±0.07) | 1.55 (±0.05) | 0.0585 (±0.0276) | 0.573 (±0.0037) | 0.0115 (±0.0030) | 0.88 (±0.04) | 4001.5 (±196.8) |
| 3,4 benzpyrene | 6 | 0.84 (±0.10) | 2.48 (±0.31) | 3.25 (±0.63 | 1.98 (±0.19) | 0.0421 (±0.0115) | 0.0299 (±0.0065) | 0.0184* (±0.0013) | 1.49** (±0.06) | 2378.2** (±104.8) |
| Phenobarbitone | 6 | 0.83 (±0.07) | 1.61 (±0.14) | 1.93 (±0.20) | 1.39 (±0.09) | 0.0252 (±0.0064) | 0.0497 (±0.0144) | 0.0222** (±0.0021) | 1.85** (±0.22) | 2028.7** (±239.1) |

* $P < 0.05$
** $P < 0.01$
*** $P < 0.005$

4.6 Pharmacokinetics of tocainide given in a single oral dose in 3,4 benzpyrine and in phenobarbitone pretreated rats

3,4 benzpyrene (80 $mg.kg^{-1}$) was administered by single i.p. injection to six rats weighing 400 to 480 g and phenobarbitone was administered i.p. daily for 4 days in six rats weighing 460.to 490 g. Tocainide (50 $mg.kg^{-1}$) was administered to all rats in each group in a single dose 48 hours after 3,4 benzpyrene and 24 hours after the last dose of phenobarbitone. Blood samples were taken at intervals and assayed for tocainide.

The concentration-time data for individual animals and the means for each gorup are presented in Appendix 9. The group of six rats which also received tocainide 50 $mg.kg^{-1}$ by single oral dose but without prior administration of 3,4 benzpyrene or phenobarbitone in the earlier dose-ranging study (Chapter 3, 4) served as a control group in this study. The mean blood concentrations in control and in enzyme-induced rats have been plotted on a linear scale (Figure 4.12) and on a logarithmic scale (Figure 4.13) aginst time. The kinetic parameters of each group were derived as described in Appendix 20 and the mean data are listed in Table 4.11. The groups were compared by student's 't' test and by Wilcoxon signed rank test. No difference was found in the rate constants for drug absorption ($k_a$) but pretreatment with both 3,4 benzpyrene and with phenobarbitone was associated with an increase in the disposition rate constant $k_d$. Consequently $t_{\frac{1}{2}}$ was reduced in both enzyme-induced groups. The $AUC_{p.o.}$ was also considerably reduced by pretreatment with both 3,4 benzpyrene (49.1%) and with phenobarbitone (57.5%). Consequently the oral clearance of tocainide was significantly increased ($p<0.01$) in both groups of rats which had received enzyme-inducing drugs. Change in $t_{\frac{1}{2}}$, AUC and oral clearance of tocainide in control and in enzyme-induced rats are shown in Figure 4.12.

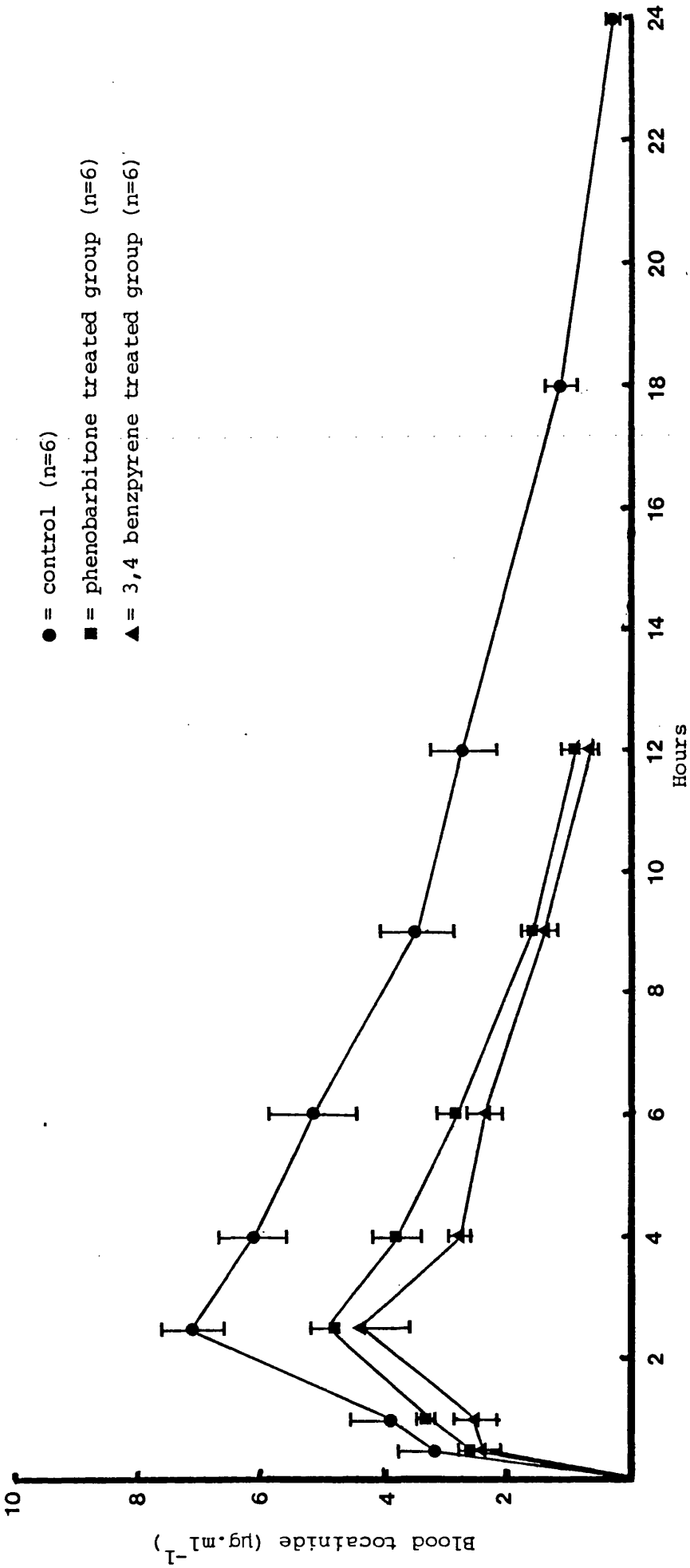



Figure 4.12 Blood concentration-time graphs of tocainide ($50mg.kg^{-1}$) following a single oral dose in phenobarbitone and in 3,4 benzpyrene treated rats. (Mean ± S.E.M.)

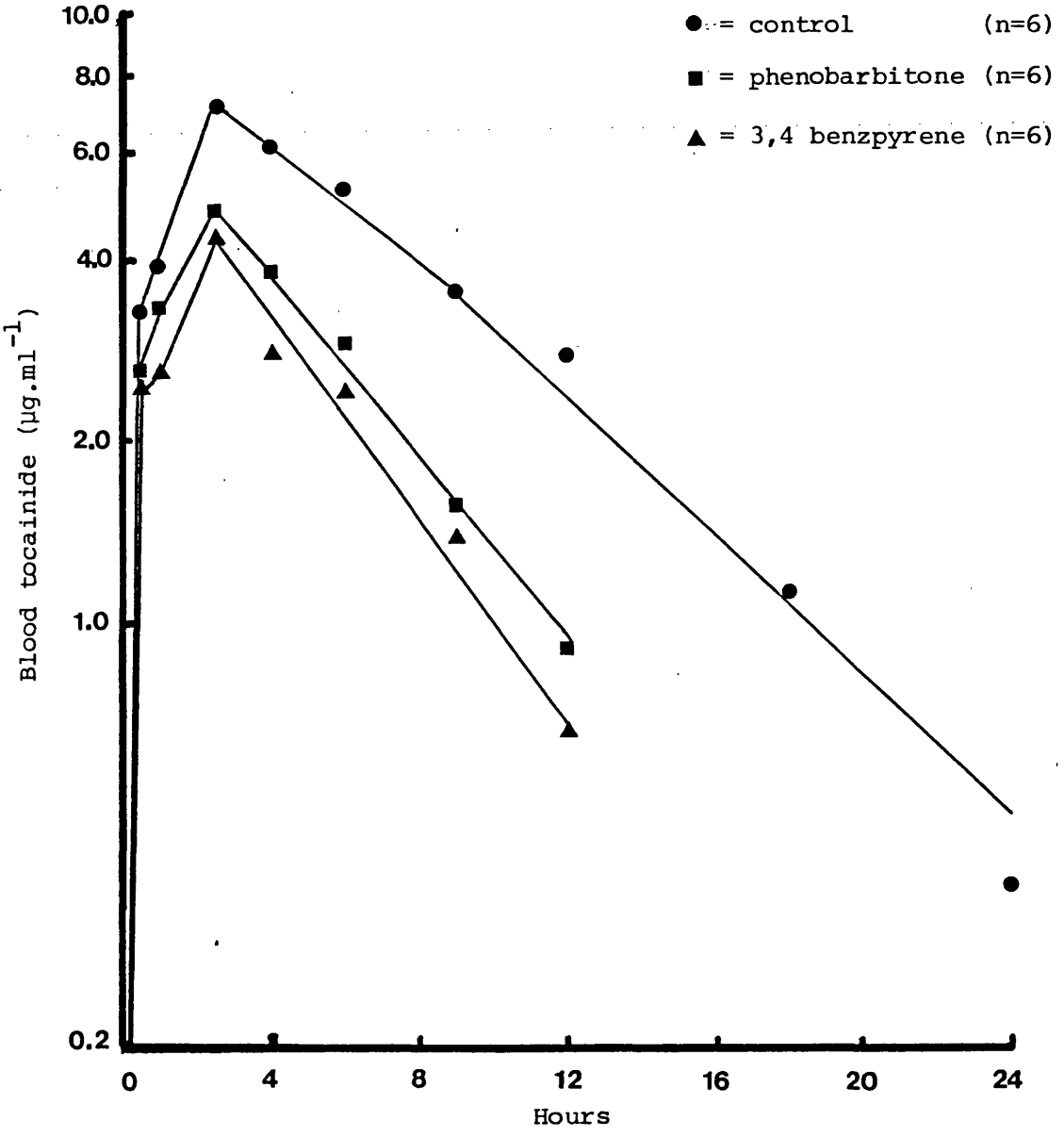



Figure 4.13 Mean blood concentration-time profiles of tocainide (50mg.kg$^{-1}$) following single oral administration in phenobarbitone and 3,4 benzpyrene treated rats.

Table 4.11 Pharmacokinetic parameters of tocainide following a single oral dose (50 $mg.kg^{-1}$) in control, 3,4 benzpyrene and phenobarbitone treated rats

| | control | 3,4 benzpyrene | phenobarbitone |
|---|---|---|---|
| n | 6 | 6 | 6 |
| Body weight (g) | 524.2 ±14.2 | 428.3±13.0 | 475.0±8.9 |
| $k_a$ ($min^{-1}$) | 0.0090 ±0.0023 | 0.0119±0.0016 | 0.0134±0.003 |
| $k_d$ ($min^{-1}$) | 0.0026 ±0.0002 | 0.0033±0.0002† | 0.0033±0.0001* |
| $t_{\frac{1}{2}}$ (hr) | 4.6±0.3 | 3.5±0.16* | 3.5±0.10* |
| $AUC_{po}$ ($\mu g.ml^{-1}.min$) | 4303.01 ±544.32 | 2190.45±146.08** | 1827.48±167.50*** |
| CL ($ml.min^{-1}.100g,bw^{-1}$) | 1.3±0.2 | 2.4±0.2** | 2.9±0.32** |

† $p<0.05$

* $p<0.02$

** $p<0.01$

*** $p<0.002$

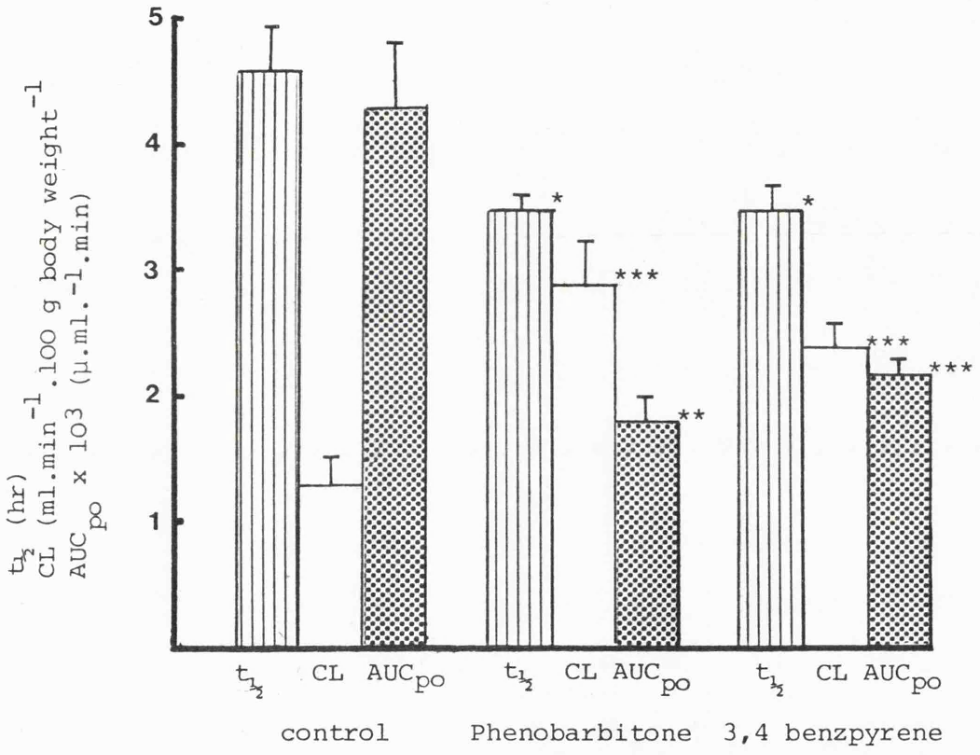



Figure 4.12 Effects of enzyme inducing drugs on half-life, clearance and area under blood concentration-time curve of tocainide following a single oral dose (50 mg.kg$^{-1}$)

* $p<0.02$

** $p<0.002$

*** $p<0.01$

# CHAPTER V

## Effects of alteration in liver blood flow by sotalol on the pharmacokinetics of lignocaine and of tocainide in rat

In this Chapter the effects on the pharmacokinetics of lignocaine and of tocainide brought about by alteration in blood flow by the beta-adrenoceptor antagonist, sotalol are presented.

## 5.1 Effects of sotalol on mean arterial pressure and on cardiac output

Preliminary dose ranging expreiments with sotalol were undertaken. Blood pressure and heart rate were recorded in pentobarbitone anaesthetized rats which had received sotalol in doses varying between 10 $mg.kg^{-1}$ and 300 $mg.kg^{-1}$. The objective of these experiments was to find a dosing regime which would acheive a significant and stable reduction in LBF sufficient to study the kinetics of single doses of lignocaine and tocainide under conditions of reduced blood flow. No significant difference in either blood pressure or in heart rate in rats were noted with doses of sotalol from 10 to 50 $mg.kg^{-1}$, i.p.. Table 5.1 shows the effect of sotalol at higher dosage levels on mean arterial pressure, heart rate and cardiac output in anaesthetized rats. A dose of 100 $mg.kg^{-1}$, i.p. was noted to being about a satisfactory fall in heart rate and arterial pressure for 3 hours. At doses in excess of this, rats died after 2 hours. The following dosing regimen was therefore adopted; sotalol 100 $mg.kg^{-1}$, i.p., was administered at -24 hours, at-18 hours, at-12 hours before either lignocaine and tocainide, then at 0 hours followed by either lignocaine 15 minutes later or tocainide 3o minutes later. In experiments involving lignocaine no further sotalol was given but in experiments involving tocainide, sotalol (100 $mg.kg^{-1}$) was given again at+4 hours, at+8 hours and at+12 hours after dosing with tocainide.

Table 5.1 Effects of a single intraperitoneal doses of sotalol on mean arterial pressure and on heart rate in pentobarbitone anaesthetized rats

| Sotalol dose | n | Heart rate ($min^{-1}$) | | | | | Mean Arterial pressure (mm.Hg) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| ($mg.kg^{-1}$) | | 0.5hr | 1hr | 2hr | 3hr | 6hr | 0.5hr | 1hr | 2hr | 3hr | 6hr |
| Control | 10(m) | 440 | — | — | — | — | 116.5 | — | — | — | — |
| 75 | 2(m) | — | 360 | 360 | 380 | 400 | — | 85 | 90 | 95 | 110 |
| 100 | 2(m) | — | 340 | 330 | 360 | 400 | — | 80 | 85 | 90 | 100 |
| 200 | 1 | — | 300 | 280 | † | | — | 80 | 45 | † | |
| 300 | 1 | — | 285 | 240* | † | | — | 80 | 20 | † | |

* = developed irregular heart beat

† = animal died

m = mean values

Table 5.2 gives mean data for arterial pressure and cardiac output in rats 30 minutes after the fourth (i.e. 0 hour) dose of sotalol.

Table 5.2 Effects of sotalol on mean arterial pressure and on cardiac output in rats (for details of dosing see text; mean ± SEM)

| | Control | Sotalol |
|---|---|---|
| n | 10 | 7 |
| mean arterial pressure (mm.Hg) | 116.5 ± 3.1 | 83.2 ± 5.9* |
| Cardiac output | | |
| $ml.min^{-1}$ | 87.1 ± 9.3 | 50.5 ± 3.6** |
| $ml.min^{-1}.kg^{-1}$ | 192.4 ± 13.4 | 142.2 ± 10.7*** |

* $p<0.001$

** $p<0.01$

*** $p<0.02$

## 5.2 Effects of sotalol on liver blood flow and on liver weight

Sotalol (100 $mg.kg^{-1}$) was given by intraperitoneal injection, as described in 5.1 at -24 hours, -18 hours, -12 hours and 0 hours to seven rats weighing 245 to 415 g. Thirty minutes after the fourth dose liver blood flow was measured as described in Chapter 2. The individual values appear in Appendix 16 and the mean data are shown in Table 5.3. Ten rats weighing 300 to 595 g and which received no sotalol (Chapter 4.2) served as a control group. Hepatosplanchnic flow decreased by 29.1% after sotalol when flow is expressed "per g of liver" and 22.7% when flow is expressed per 100 g body weight. The reduction in hepatosplanchnic flow was principally accounted for by a reduction in hepatic arterial flow ($p<0.05$). Flow to GI tract and pancreas and splenic blood flow were also reduced but these differences were not significant.

Table 5.3 Effects of sotalol on liver weight and liver blood flow in pentobarbitone anaesthetized rats. (Mean ± SEM)

| | Saline | Sotalol |
|---|---|---|
| No of rats | 10 | 7 |
| Liver weights (g/100 g b.w.) | 3.48 ± 0.13 | 3.73 ± 0.19 |
| Hepatosplanchnic blood flow | | |
| $ml.min^{-1}$ g $liver^{-1}$ | 1.17 ± 0.11 | 0.83 ± 0.09* (t=2.214) |
| $ml.min^{-1}$.100g,bw-1 | 4.06 ± 0.43 | 3.14 ± 0.34 |
| GI tract and pancreas blood flow[a] | | |
| weight (g) | 13.5 ± 1.1 | 12.4 ± 0.8 |
| $ml.min^{-1}$ g $tissue^{-1}$ | 1.05 ± 0.15 | 0.67 ± 0.08 |
| $ml.min^{-1}$.100g,$bw^{-1}$ | 2.70 ± 0.30 | 2.33 ± 0.33 |
| Hepatic arterial flow[b] | | |
| $ml.min^{-1}$,g $liver^{-1}$ | 0.30 ± 0.05 | 0.14 ± 0.02* (t=2.390) |
| $ml.min^{-1}$,100g,$bw^{-1}$ | 1.04 ± 0.20 | 0.51 ± 0.08* (t=2.142) |
| Spleen blood flow | | |
| weight (g) | 0.9 ± 0.04 | 0.8 ± 0.05 |
| $ml.min^{-1}$,g $tissue^{-1}$ | 1.87 ± 0.25 | 1.13 ± 0.26 |
| $ml.min^{-1}$.100g,$bw^{-1}$ | 0.32 ± 0.03 | 0.26 ± 0.06 |

NB $t = 2.131$, $df = 15$, $p<0.05$

a = portal flow

b = hepatic artery flow

a+b = hepatosplanchnic flow

Table 5.4 Effect of sotalol on blood flow to heart, lungs and kidneys in rat. (Mean ± SEM)

| | Saline | Sotalol |
|---|---|---|
| n | 10 | 7 |
| **Heart** | | |
| weight (g) | 1.68 ± 0.12 | 1.30 ± 0.09 |
| blood flow | | |
| $ml.min^{-1}.g\ tissue^{-1}$ | 2.24 ± 0.46 | 2.03 ± 0.26 |
| $ml.min^{-1}.100g,b.w.^{-1}$ | 0.82 ± 0.13 | 0.73 ± 0.10 |
| **Lungs** | | |
| weight (g) | 1.92 ± 0.12 | 2.36 ± 0.27 |
| blood flow | | |
| $ml.min^{-1}.g\ tissue^{-1}$ | 0.67 ± 0.11 | 0.55 ± 0.32 |
| $ml.min^{-1}.100g,b.w.^{-1}$ | 0.26 ± 0.05 | 0.28 ± 0.14 |
| **Kidneys** | | |
| weight (g) | 3.74 ± 0.15 | 3.07 ± 0.16 |
| blood flow | | |
| $ml.min^{-1}.g\ tissue^{-1}$ | 4.35 ± 0.66 | 3.27 ± 0.39 |
| $ml.min^{-1}.100g,b.w.^{-1}$ | 3.22 ± 0.50 | 2.75 ± 0.31 |

No change in the weight of liver, spleen or in the combined weight of pancreas plus GI tract was noted after administration of sotalol.

## 5.3 Effects of sotalol on blood flow to heart, lungs and kidneys and percentage of cardiac output received by major organs

The individual values for blood flow to heart, lungs and kidneys and for fractional distribution of cardiac output appear in Appendix 16. Blood flow to heart and kidneys was slightly reduced after administration of sotalol but these changes were not significant. No change was noted in the weights of these organs (Table 5.4). Percentage of cardiac output received by major organs in control and sotalol treated animals are listed in Table 5.5. There were no significant changes in the proportion of the cardiac output distributed to these organs.

Table 5.5 Effect of sotalol on percentage of cardiac output received by major organs in rats. (Mean ± S.E.M.)

| | Saline | Sotalol |
|---|---|---|
| n | | |
| Hepatosplanchnic | 24.0 ± 1.5 | 20.7 ± 0.7 |
| GI tract and pancreas | 15.9 ± 1.3 | 15.5 ± 1.1 |
| Liver | 6.2 ± 1.0 | 3.6 ± 0.6 |
| Spleen | 1.9 ± 0.1 | 1.7 ± 0.3 |
| Lungs | 1.4 ± 0.3 | 1.8 ± 0.9 |
| Kidneys | 18.9 ± 2.1 | 18.8 ± 1.4 |
| Heart | 4.8 ± 0.6 | 4.8 ± 0.3 |

## 5.4 Pharmacokinetics of lignocaine following a single intravenous dose in rats pretreated with sotalol

Sotalol (100 $mg.kg^{-1}$, i.p.) was administered to six rats weighing 360 to 410 g, as described in 5.1 at -24 hours, -18 hours, -12 hours and 0 hours. Fifteen minutes after the fourth dose of sotalol, lignocaine (5 $mg.kg^{-1}$) was administered by intravenous injection into the tail of each rat. Blood samples were taken at appropriate times and assayed for lignocaine.

The blood concentration-time data for each rat and the means for the group are given in Appendix 2. The mean blood concentration-time data are plotted on a linear scale (Figure 5.1) and on a logarithmic scale (Figure 5.2) against time. Kinetic parameters were obtained from the blood concentration-time data for each animal using the non-linear least squares regression analysis programme NONLIN as described in Appendix 21 and the mean values appear in Table 5.6. The data in the treated group are compared with those in eight rats which also received lignocaine 5 $mg.kg^{-1}$, i.v., as part of the earlier dose ranging study (Chapter 3.1). These rats had not been pretreated with sotalol and therefore served as a control group. Table 5.6 shows that the rate constants for the slow ($\beta$) phase of decline in blood lignocaine concentration with time was significantly decreased ($p<0.05$ by 't' test) in the sotalol treated group; consequently $t_{\frac{1}{2}\beta}$ was significantly prolonged in this group (78.5 min) as compared with the control (48.1 min). Table 5.6 also shows that sotalol pretreatment was associated with an approximate doubling of AUC, consequently systemic clearance of lignocaine was approximatley halved in the sotalol pretreated group. No statistically significant changes were noted in other kinetic parameters.

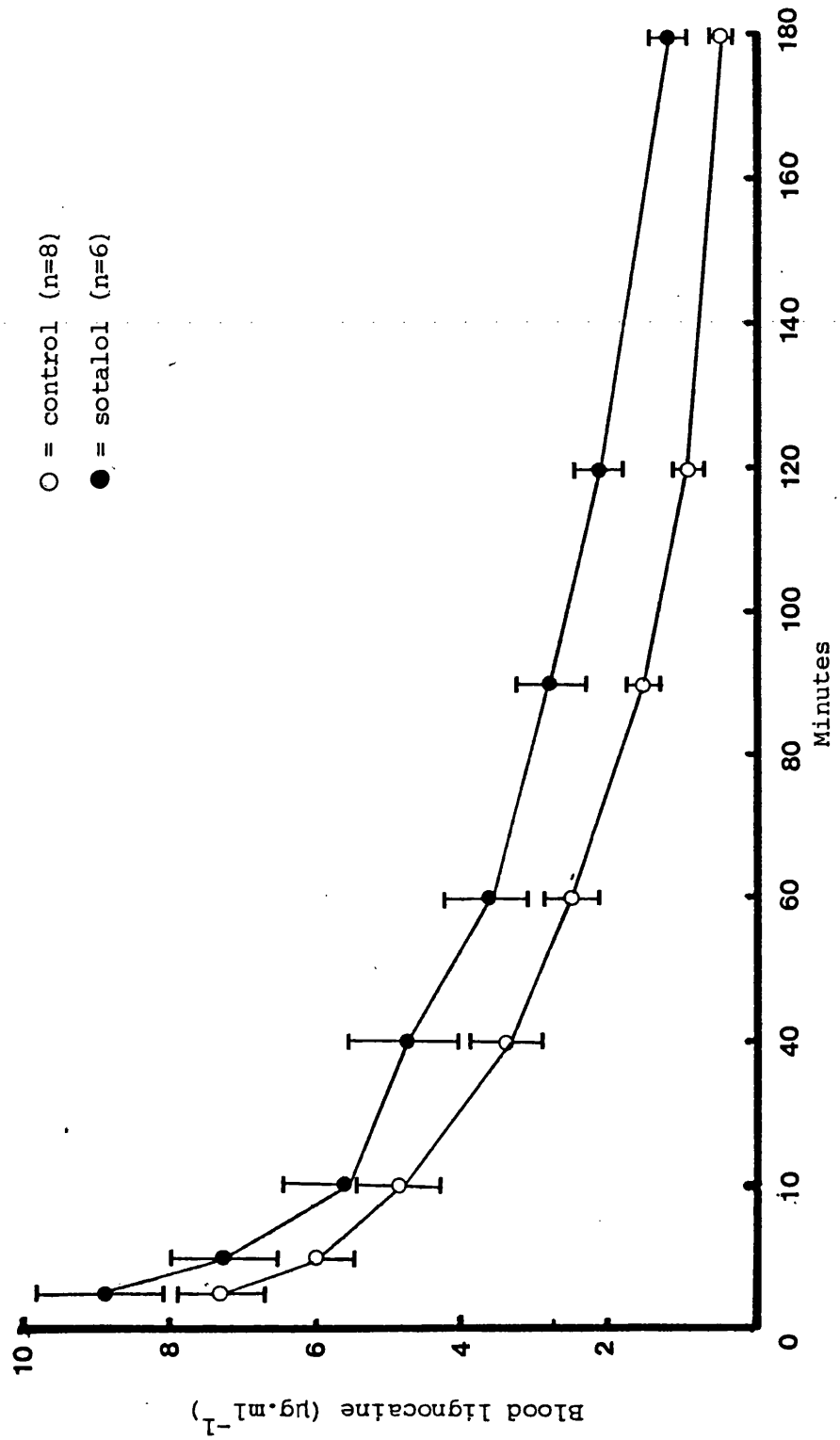



Figure 5.1 Blood concentration-time graphs following lignocaine 5 mg.kg, i.v. in control and in sotalol treated rats. (Mean ± SEM)

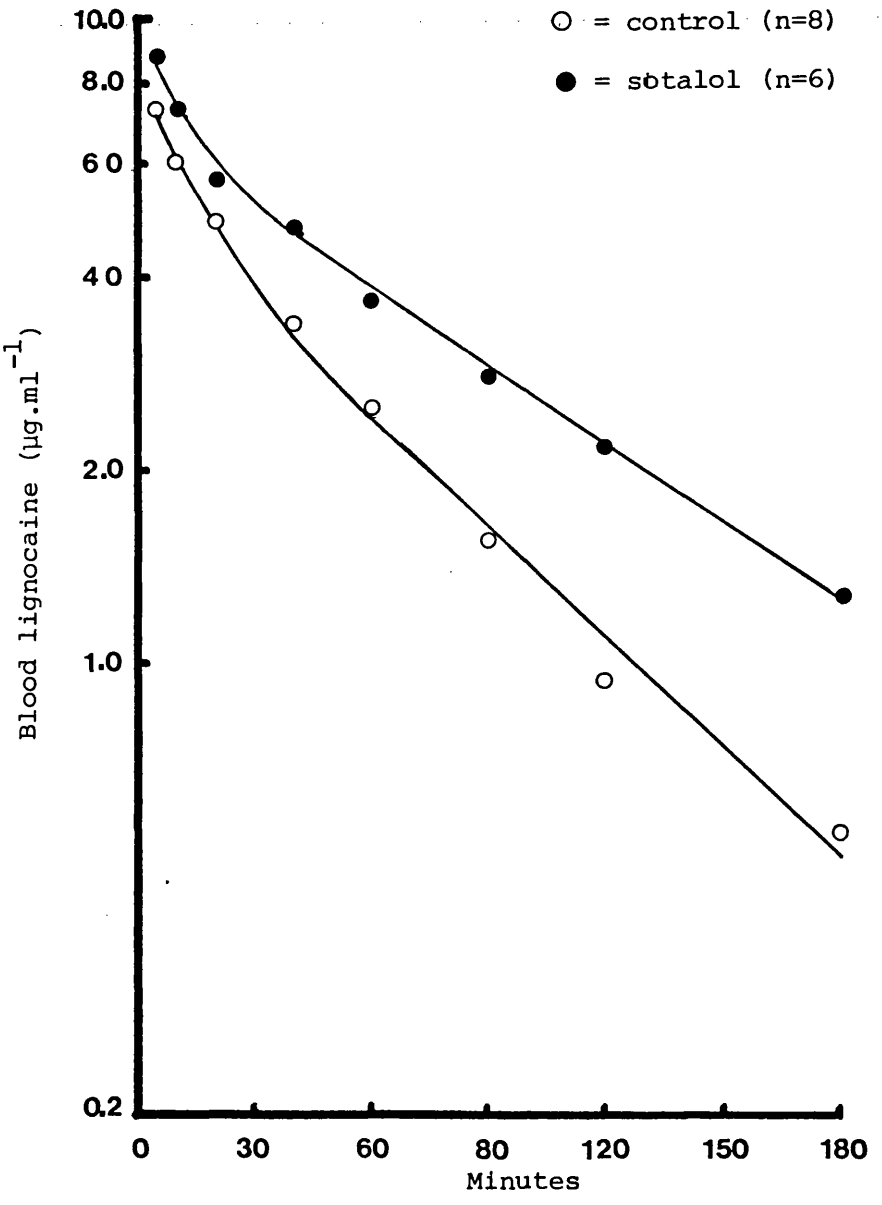



Figure 5.2 Mean blood concentration-time graphs following lignocaine 5 mg.kg$^{-1}$ in control and in sotalol pretreated rats

Table 5.6 Pharmacokinetic parameters of lignocaine following 5 $mg.kg^{-1}$, i.v. in control and in sotalol pretreated rats

| Treatment | n | Body weight (g) | A ($\mu g.ml^{-1}$) | B ($\mu g.ml^{-1}$) | $\alpha$ ($min^{-1}$) | $\beta$ ($min^{-1}$) | $t_{\frac{1}{2}\alpha}$ (min) | $t_{\frac{1}{2}\beta}$ (min) |
|---|---|---|---|---|---|---|---|---|
| Control | 8 | 394.4 (±5.1) | 7.2 (±1.7) | 5.9 (±0.9) | 0.2208 (±0.0642) | 0.0148 (±0.0009) | 6.8 (±2.5) | 48.1 (±3.9) |
| Sotalol | 6 | 385.8 (±7.3) | 7.9 (±1.2) | 7.0 (±0.8) | 0.3172 (±0.1190) | 0.091* (±0.0007) | 5.2 (±1.4) | 78.5* (±7.7) |

| Treatment | n | Vp ($l.kg^{-1}$) | $Vd_{area}$ ($l.kg^{-1}$) | $Vd_{extrap}$ ($l.kg^{-1}$) | $Vd_{ss}$ ($l.kg^{-1}$) | $k_{12}$ ($min^{-1}$) | $k_{21}$ ($min^{-1}$) | $k_e$ ($min^{-1}$) | Total body clearance ($ml.min^{-1}.100g,bw^{-1}$) | AUC ($\mu g.ml^{-1}.min$ |
|---|---|---|---|---|---|---|---|---|---|---|
| Control | 8 | 0.48 (±0.07) | 0.84 (±0.10) | 1.01 (±0.19) | 0.76 (±0.08) | 0.1119 (±0.0914) | 0.1101 (±0.0252) | 0.0275 (±0.0034) | 1.21 (±0.12) | 444.7 (±48.2) |
| Sotalol | 6 | 0.35 (±0.04) | 0.71 (±0.08) | 0.77 (±0.09) | 0.65 (±0.06) | 0.0798 (±0.0192) | 0.0942 (±0.0227) | 0.0188 (±0.0024) | 0.63*** (±0.49) | 822.8** (±65.5) |

* $p<0.05$

** $p<0.001$

*** $p<0.002$

## 5.5 Pharmacokinetics of lignocaine following a single oral dose in sotalol pretreated rats

Sotalol (100 $mg.kg^{-1}$) was administered to six rats, weighing 355 to 425 g, by intraperitoneal injection as described in 5.1 at -24 hours, -18 hours, -12 hours and at 0 hours. Fifteen minutes after the fourth dose of sotalol, lignocaine (70 $mg.kg^{-1}$) was administered by mouth to each rat. Blood samples were taken at appropriate times and assayed for lignocaine.

The blood concentration-time data for each rat and the means for the group are given in Appendix 4. The mean blood concentration-time data are plotted on a linear scale (Figure 5.3) and on logarithmic scale (Figure 5.4) against-time. Kinetic parameters were derived from the blood concentration-time data for each rat as described in Appendix 20 and the mean values are given in Table 5.7. The data was compared with those in eight rats which had also received lignocaine 70 $mg.kg^{-1}$ by mouth as part of the earlier dose ranging study (Chapter 3.2). These rats had not been pretreated with sotalol and therefore served as a control group. Table 5.7 shows that after sotalol there was no significant change in the rate constant for absorption ($k_a$) but that there was a significant decrease ($p<0.001$ by student's 't' test) in the disposition rate constant ($k_d$). Consequently $t_{\frac{1}{2}}$ was lengthened significantly in the sotalol treated group. Furthermore AUC was approximately doubled by sotalol pretreatment ($p<0.01$) and in consequence the apparent oral clearance of lignocaine was almost half of the value for the control rats.

Changes in $t_{\frac{1}{2}}$, CL and AUC for control and for sotalol pretreated rats are given in Figure 5.5.

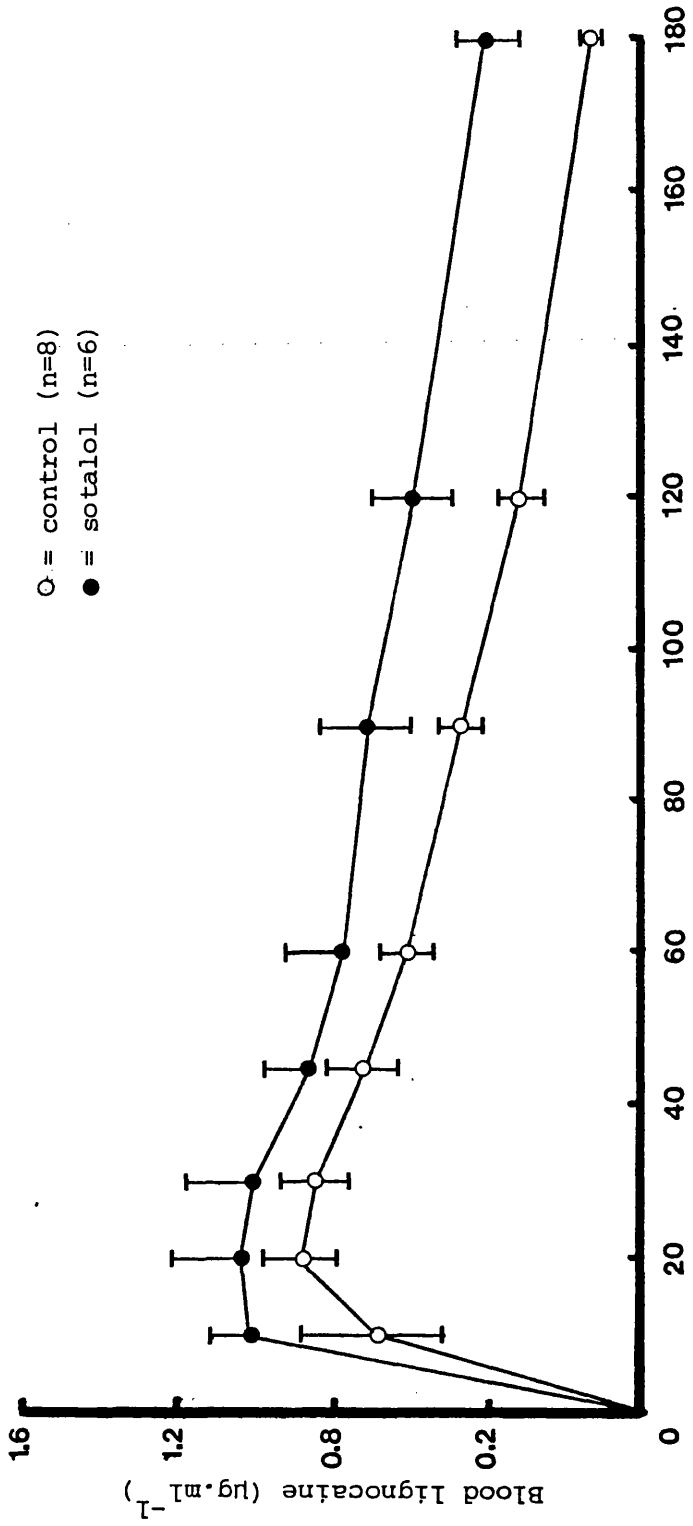



Figure 5.3 Mean blood concentration-time graphs following lignocaine (70 mg.kg$^{-1}$ by mouth) in control and in sotalol treated rats. (Mean ± SEM)

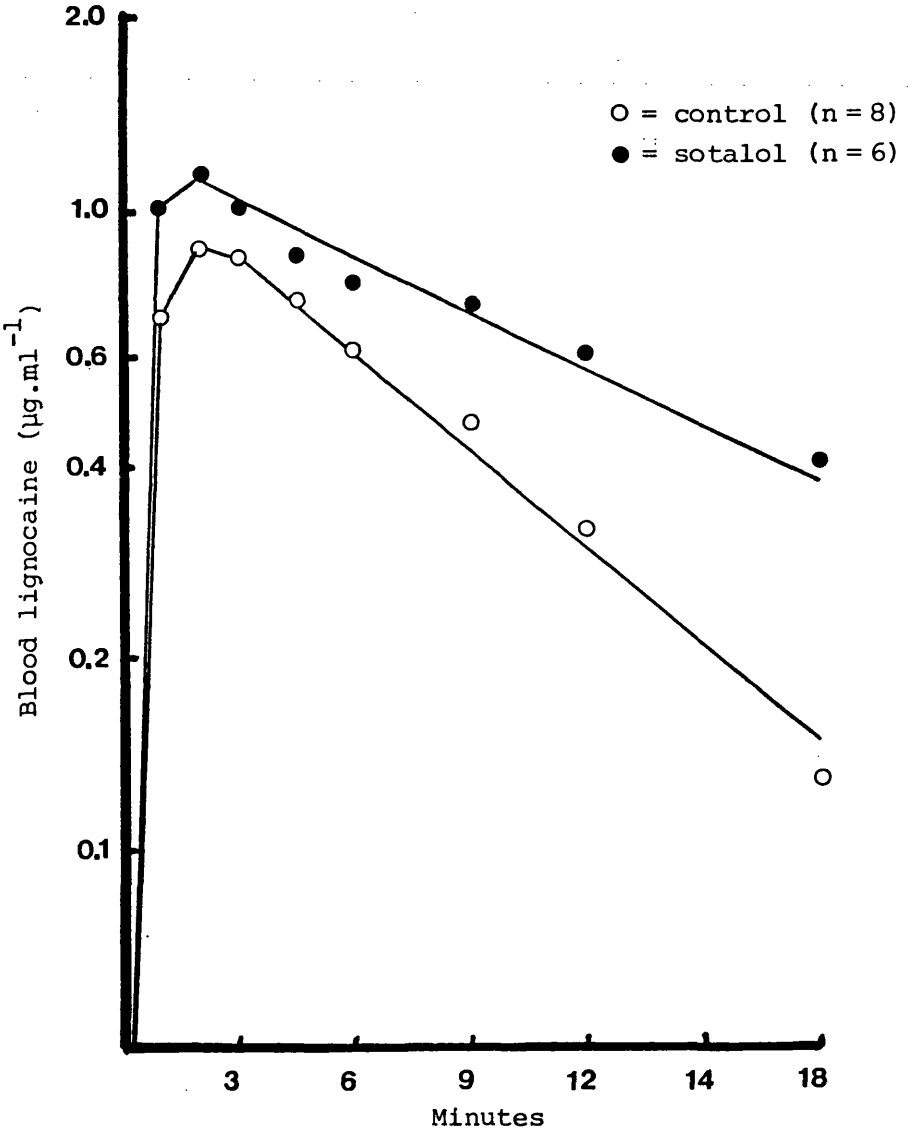



Figure 5.4 Mean blood concentration-time graphs following lignocaine (70 mg.kg$^{-1}$ by mouth) in control and in sotalol treated rats

Table 5.7 Pharmacokinetic parameters of lignocaine following 70 mg.kg$^{-1}$ by mouth)in control and in sotalol treated rats (Mean ± SEM)

| | Control | Sotalol |
|---|---|---|
| n | 8 | 6 |
| Body weight (g) | 417.5 ± 13.2 | 396.7 ± 9.5 |
| $k_a$ ($min^{-1}$) | 0.1044 ± 0.0174 | 0.1027 ± 0.0242 |
| $k_d$ ($min^{-1}$) | 0.0121 ± 0.0009 | 0.0054 ± 0.0005** |
| $t_{\frac{1}{2}}$ (min) | 59.2 ± 4.6 | 135.4 ± 16.1** |
| $AUC_{p.o.}$ ($\mu g.ml^{-1}.min$) | 103.10 ± 9.48 | 205.80 ± 27.78* |
| CL ($ml.min^{-1}.100g,bw^{-1}$) | 72.97 ± 7.98 | 36.74 ± 4.10* |

* $p<0.01$

** $p<0.001$

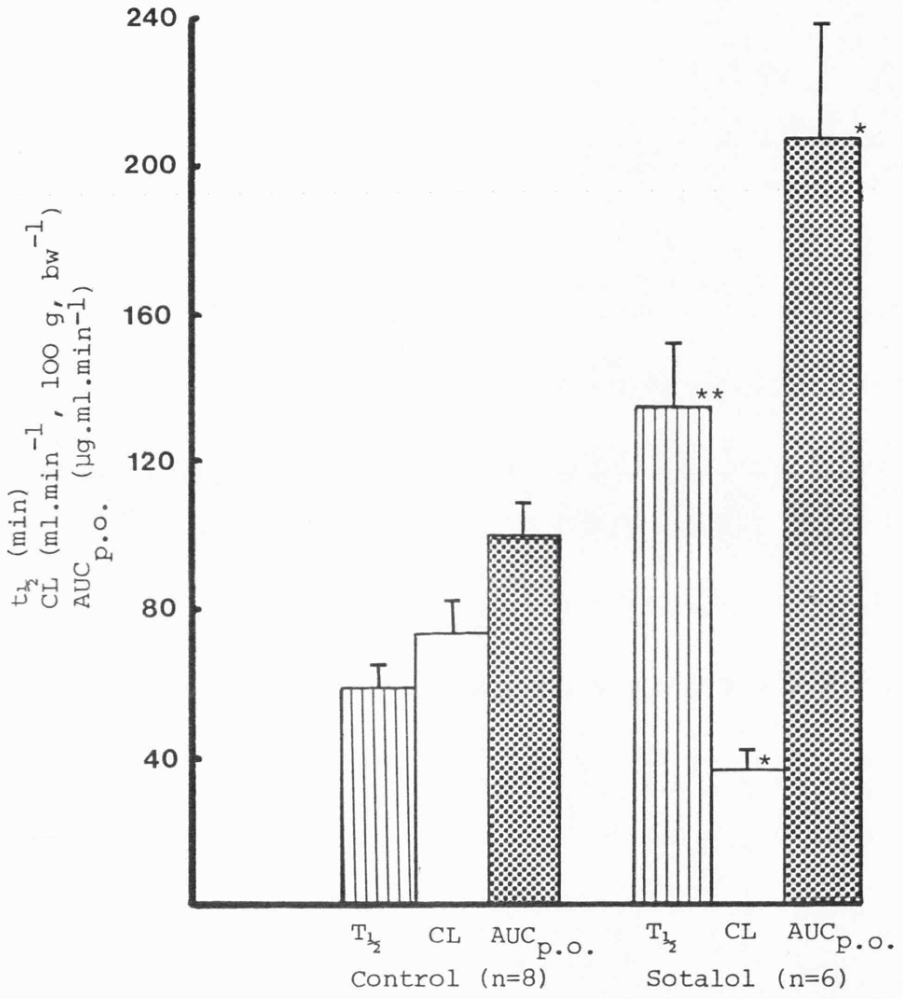



Figure 5.5 Effects of sotalol on half-life, apparent oral clearance and area under blood concentration-time curve of lignocaine (70 mg.kg$^{-1}$) by mouth

* $p<0.01$

** $p<0.001$

5.6 Pharmacokinetics of tocainide following a single intravenous dose in rats pretreated with sotalol

Sotalol (100 $mg.kg^{-1}$) was administered to six rats, weighing 350 to 490 g, i.p. at -24 hours, -18 hours, -12 hours and 0 hours as described in 5.1. Thirty minutes after the fourth dose of sotalol, tocainide (35 $mg.kg^{-1}$) was administered by intravenous injection into the tail vein of each rat. Sotalol (100 $mg.kg^{-1}$, i.p.) was also given +4 hours, +8 hours and +12 hours. Blood samples were taken at appropriate times and assayed for tocainide.

The blood concentration-time data for each rat and the means for the group are given in Appendix 6. The mean blood concentration-time data are plotted on a linear scale (Figure 5.6) and on a logarithmic scale (Figure 5.7) against time. Kinetic parameters were obtained from the blood concentration-time data for each animal using the non-linear least squares regression analysis programme NONLIN as described in Appendix 21 and the mean values appear in Table 5.8. The treated group are compared with the data in five rats which also received tocainide 35 $mg.kg^{-1}$, i.v., as part of the earlier dose ranging study (Chapter 3.3). These rats had not been pretreated with sotalol and therefore serve as a control group. Table 5.8 shows that sotalol treatment caused no significant changes in the pharmokinetic parameters of tocainide.

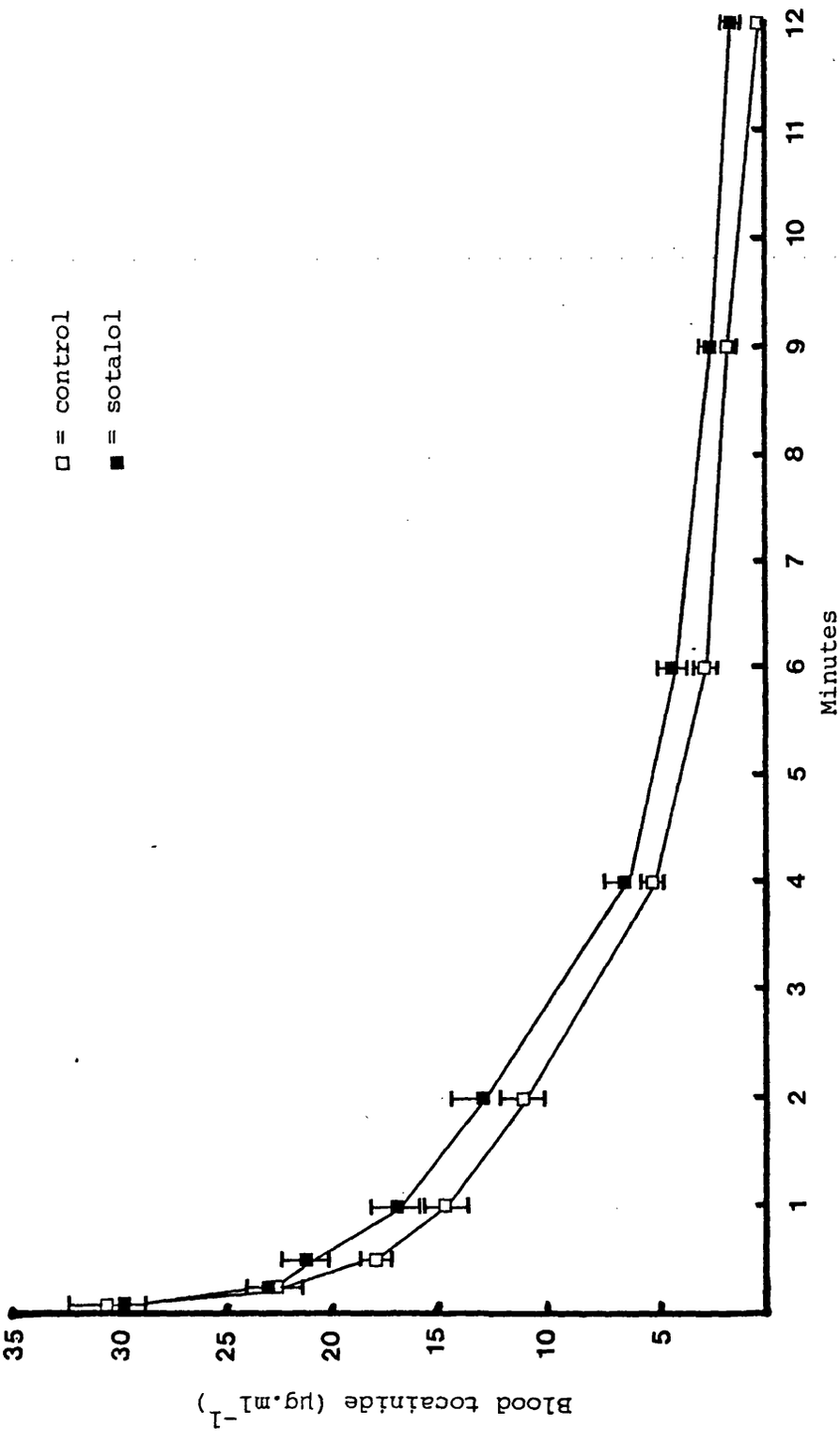



Figure 5.6 Blood concentration-time graphs of tocainide following 35 mg.kg, i.v., in control and in sotalol treated rats. (Mean ± SEM)

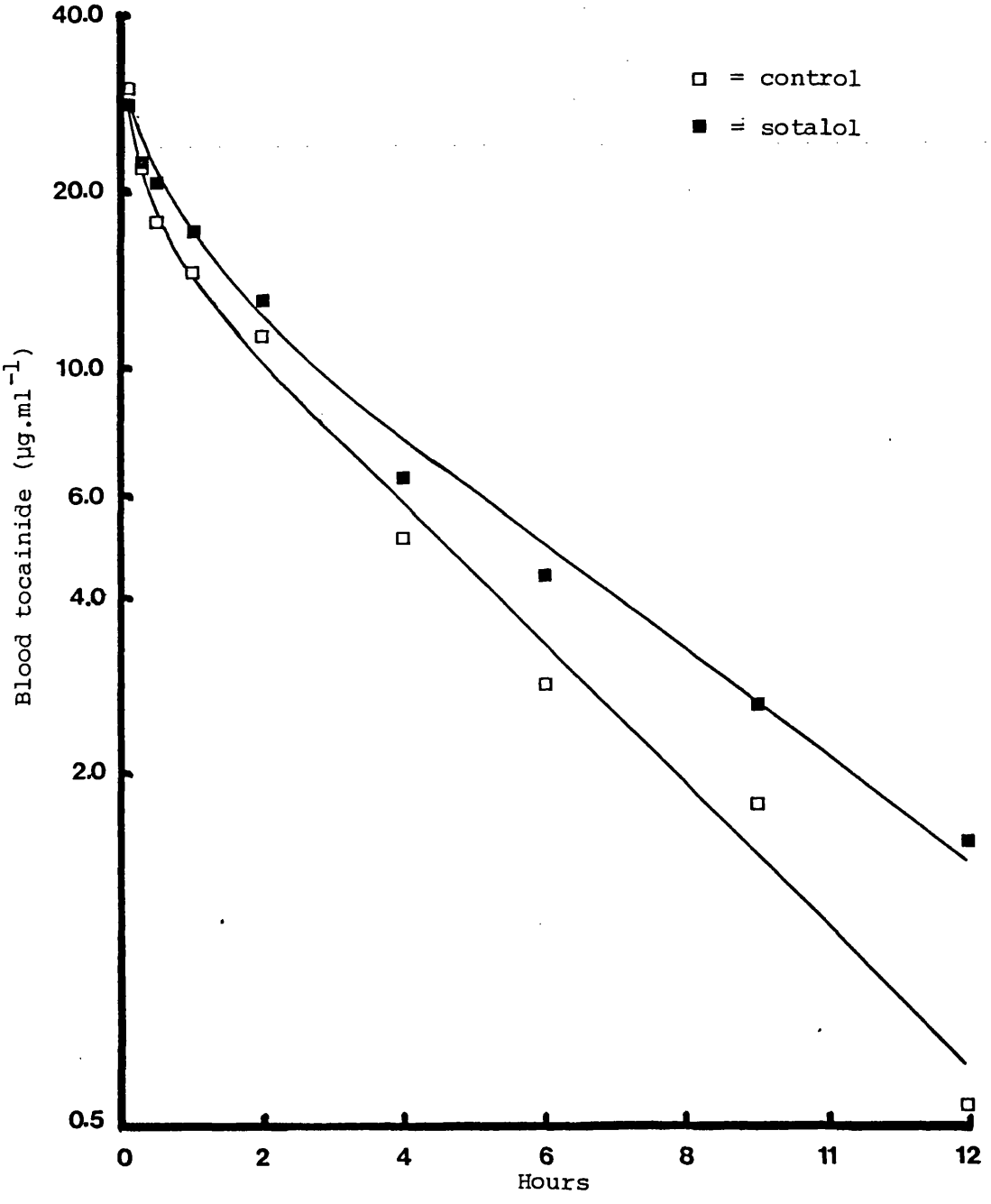



Figure 5.7 Blood concentration-time graphs following tocainide 35 mg.kg$^{-1}$, i.v. in control and in sotalol pretreated rats

Table 5.8 Pharmacokinetic parameters of tocainide following 35 $mg.kg^{-1}$, i.v. in control and in sotalol pretreated rats

| Treatment | n | Body weight (g) | A ($\mu g.ml^{-1}$) | B ($\mu g.ml^{-1}$) | $\alpha$ ($min^{-1}$) | $\beta$ ($min^{-1}$) | $t_{\frac{1}{2}\alpha}$ (min) | $t_{\frac{1}{2}\beta}$ (min) |
|---|---|---|---|---|---|---|---|---|
| Control | 5 | 518.0 (±15.9) | 23.5 (±9.6) | 20.8 (±0.9) | 0.1219 (±0.0325) | 0.0054 (±0.0002) | 7.0 (±1.3) | 127.9 (±4.9) |
| Sotalol | 6 | 384.2 (±21.8) | 18.6 (±3.9) | 22.5 (±1.5) | 0.1803 (±0.0469) | 0.0047 (±0.0005) | 5.1 (±1.1) | 157.1 (±18.5) |

| Treatment | n | Vp ($l.kg^{-1}$) | $Vd_{area}$ | $Vd_{extrap}$ ($l.kg^{-1}$) | $Vd_{ss}$ ($l.kg^{-1}$) | $k_{12}$ ($min^{-1}$) | $k_{21}$ ($min^{-1}$) | $k_e$ ($min^{-1}$) | Total body clearance ($ml.min^{-1}.100g,bw^{-1}$) | AUC ($\mu g.ml^{-1}.min$) |
|---|---|---|---|---|---|---|---|---|---|---|
| Control | 5 | 0.89 (±0.13) | 1.62 (±0.06) | 1.70 (±0.07) | 1.55 (±0.05) | 0.0585 (±0.0276) | 0.0573 (±0.0037) | 0.0115 (±0.0030) | 0.88 (±0.04) | 4001.5 (±196.8) |
| Sotalol | 6 | 0.89 (±0.09) | 1.55 (±0.10) | 1.59 (±0.11) | 1.51 (±0.10) | 0.0771 (±0.0278) | 0.0993 (±0.0218) | 0.0085 (±0.0014) | 0.71 (±0.07) | 5133.0 (±502.9) |

5.7 Pharmacokinetics of tocainide following a single oral dose in sotalol pretreated rats

Sotalol (100 $mg.kg^{-1}$) was administered to six rats weighing 380 to 480 g, i.p., as described in 5.1 at -24 hours, -18 hours, -12 hours and 0 hour. Thirty minutes after the fourth dose of sotalol, tocainide (50 $mg.kg^{-1}$) was administered by mouth to each rat. Sotalol (100 $mg.kg^{-1}$, i.p.) was also given at +4 hours, +8 hours and +12 hours. Blood samples were taken at appropriate times and assayed for tocainide.

The blood concentration-time data for each rat and the means for the group are given in Appendix 4. The mean blood concentration-time data are plotted on a linear scale (Figure 5.8) and on logarithmic scale (figure 5.9) against time. Kinetic parameters were derived from the blood concentration-time data for each rat as described in Appendix 20 and the mean values are given in Table 5.9. The data are compared with those in six rats which had also received tocainide 50 $mg.kg^{-1}$ by mouth as part of the earlier dose ranging study (Chapter 3.4). These rats had not been pretreated with sotalol and therefore served as a control group. Table 5.9 shows that after sotalol there was an increase in the rate constant for absorption ($k_a$) which just achieved statistical significance ($p<0.05$) but no significant changes were noted in other pharmacokinetic parameters.

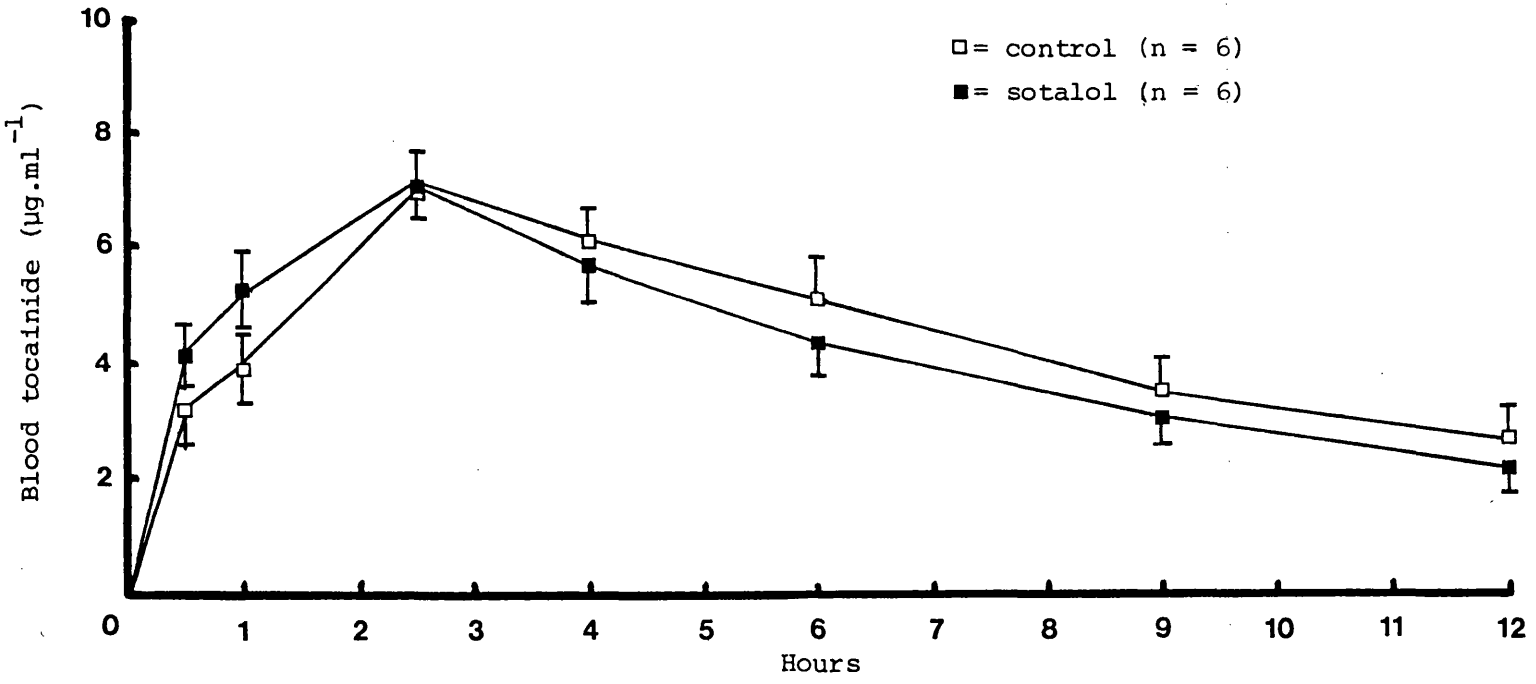



Figure 5.12 Blood concentration-time graphs of tocainide following 50 mg.kg$^{-1}$ by mouth in control and in sotalol treated rats

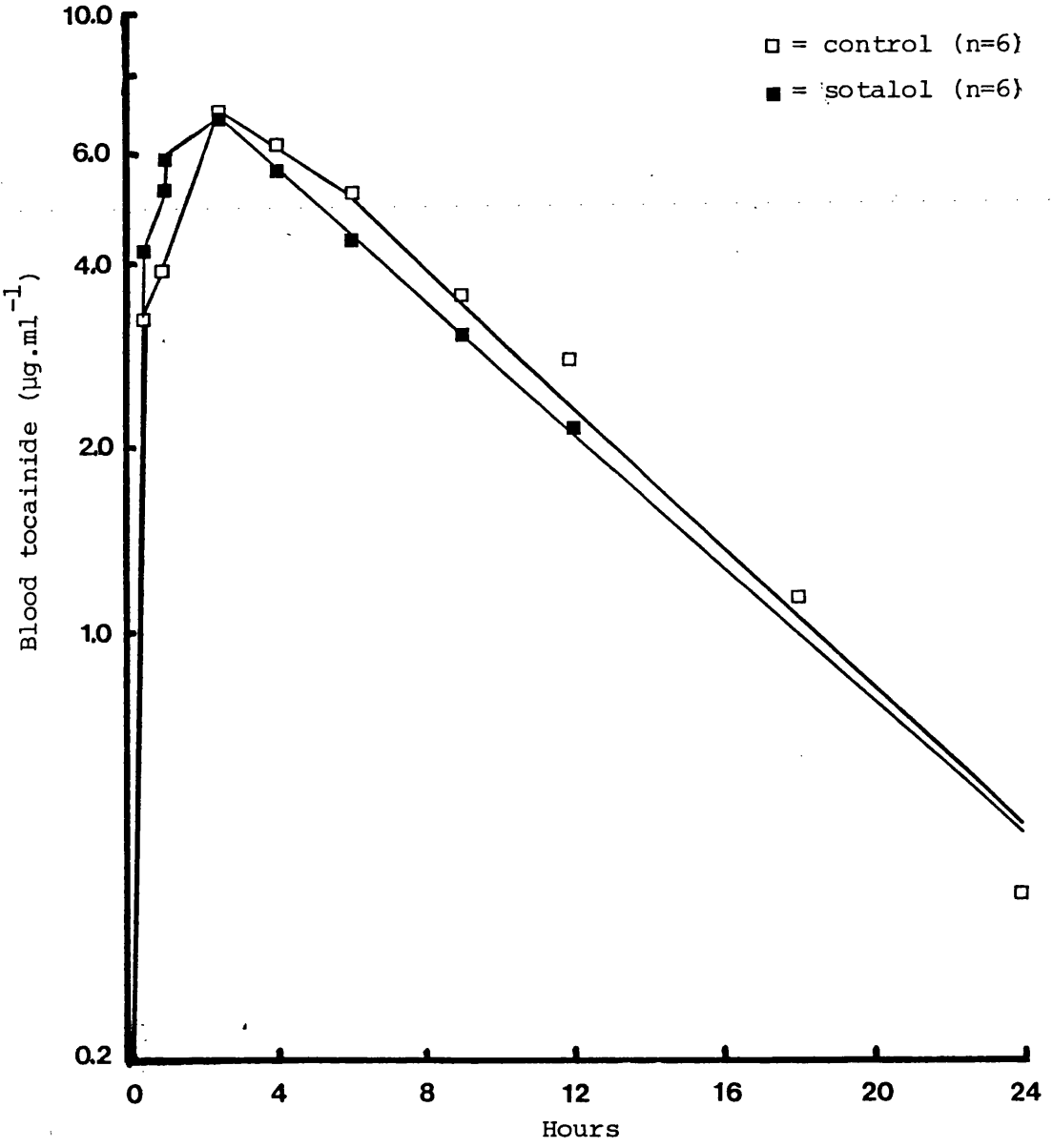



Figure 5.13 Blood concentration-time graphs of tocainide following 50 mg.kg by mouth in control and sotalol treated rats

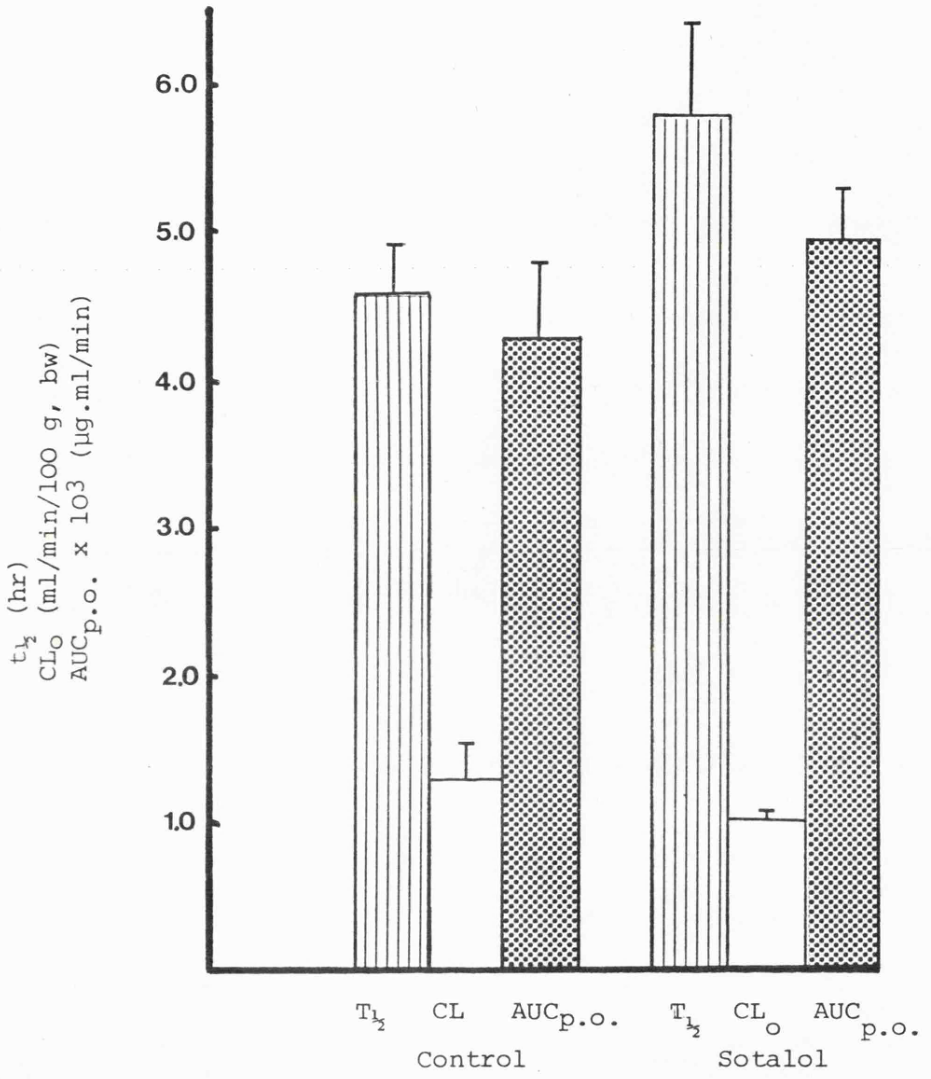



Figure 5.14 Effects of sotalol on half-life, apparent oral clearance and area under blood concentration-time curve of tocainide following 50mg.kg$^{-1}$ single oral administration

Table 5.9 Pharmacokinetic parameters of tocainide following 50mg.kg$^{-1}$ by mouth in control and in sotalol pretreated rats

| | Control | Sotalol |
|---|---|---|
| n | 6 | 6 |
| Body weight (g) | 524.2±14.2 | 402.5±6.8 |
| $k_a$ ($min^{-1}$) | 0.0090±0.0023 | 0.0240±0.0065* |
| $k_d$ ($min^{-1}$) | 0.0026±0.002 | 0.0021±0.0003 |
| $t_{\frac{1}{2}}$ (hr) | 4.6±0.3 | 5.8±0.6 |
| $AUC_{p.o.}$ ($\mu g\ ml^{-1}.min$) | 4303.01±544.32 | 4981.01±334.65 |
| CL ($ml.min^{-1}.100g,bw^{-1}$) | 1.30±0.23 | 1.03±0.07 |

* $p<0.05$

# CHAPTER VI

# DISCUSSION

# 6. DISCUSSION

## 6.1 INTRODUCTION

Lignocaine and tocainide are both used for the treatment of cardiac dysrhythmias in man. There are however, a number of important differences between the two drugs. Although they are structurally similar, lignocaine is a tertiary amine with strong local anaesthetic activity and tocainide is a primary amine which lacks local anaesthetic properties. Lignocaine is totally eliminated by the liver whereas to some extent tocainide is dependent on the kidney for elimination (McDevitt et al, 1976; Winkle et al, 1976). The kinetic properties of the two drugs also differ; lignocaine is highly hepatically cleared (Sung and Traunt, 1954; Hollunger, 1960a) shows low systemic availability by the oral route and has a short half life; tocainide is cleared more slowly, has high systemic availability by the oral route and has a long half life (Lalka et al, 1976). These properties are reflected in the ways the drug are used clinically, lignocaine by continous intravenous infusion (Harrison and Alderman, 1972) and tocainide by the oral route (Graffner, Conradson, Hofvendahl and Ryden, 1979). A further contrast is that lignocaine is a well established drug which has been used for 20 years and has been extensively studied whereas tocainide is a relatively new drug about which much less is known.

In recent years the major factors which determine drug elimination by the liver have been increasingly understood. They are liver blood flow, liver metabolism, drug binding and probably intrahepatic shunting. The present work has been concerned with the effects of two of these variables, namely blood flow and drug metabolism on the kinetics of lignocaine and tocainide. Most drugs which are eliminated by the liver may be separated into those in which blood flow and those for which metabolism is the major determinant of elimination. It is therefore to be anticipated that these variables would have different effects

on the kinetics of lignocaine (flow dependent) and tocainide (metabolism dependent). Furthermore these physiological varabiles were altered in a manner which could be clinically relevant, blood flow being reduced by β-adrenoceptor blockade and metabolism being increased by enzyme induction.

In the first instance therefore the work was undertaken to observe the changes which would be encountered in the kinetics of these two antiarrhythmic drugs when liver blood flow and liver metabolism were altered. However, in recent years two mathematical models have been developed in attmepts to predict drug kinetics on the basis of the major variables flow, metabolism and binding. The data in this thesis therefore, also provide an opportunity to test in part the capacity of these models to predict the kinetics of a blood-flow dependent and of a metabolism-dependent drug when factors governing elimination are altered.

## 6.2 Basic kinetics of lignocaine in the rat

The gas liquid chrmoatographic technique for measuring lignocaine has proved to be both accurate and sensitive at blood concentrations of 0.05 $\mu g.ml^{-1}$ (Chapter 2.7.3) which was adequate for the purposes of the present study. Furthermore, these estimations could be performed on small quantities of blood ($\simeq$0.35 ml). Lignocaine in blood and in urine could be stored for longer than three months prior to analysis with no measurable loss of drug. In addition the method of collecting blood, i.e. cutting off the rat tail tip proved to be sufficient for the repeated blood sampling demanded by this study.

Kinetics of lignocaine have been studied in monkey (Benowitz et al, 1974 a and b), dog (Boyes et al, 1970) and man (Boyes et al, 1971; Rowland et al, 1971; Tucker and Boas, 1971; Thomson et al, 1973; Nation et al, 1977; Perucca and Richens, 1979). The rat has also been used but as the perfused rat liver in situ (Pang and Rowland, 1977b) or as the isolated perfused rat liver (Shand, Kornhauser and Wilkinson, 1975). The present work is believed to be the first systematic attempt to investigate the basic kinetics of lignocaine in the intact rat.

The blood concentration of lignocaine in rat following intravenous injection was found to decline in a biexponential manner and may be fitted to the two compartment open model (Mayersohn and Gibaldi, 1971; Greenblatt and Koch-Weser, 1975) using graphic techniques. However, this is a tedious approach and depends on the subjective judgement of the experimenter. Therefore, the lignocaine concentration-time data were fitted to prescribed functions using the non-linear least squares regression analysis programme NONLIN (Metzler, 1969). It was found that the blood lignocaine following an intravenous dose could be adequately fitted by the sum of two exponentials and from the coefficients and exponents of the biexponential equation the volume constants and rate constants for intercompartmental transfer of drug in the two compartmental open model were derived. Table 6.1 compares the means of intravenous $AUC_{0\to\infty}$ calculated by the computer programme "NONLIN" and by trapezoidal rule indicating there were no significant differences between the means of $AUC_{0\to\infty}$ obtained by these two methods.

Table 6.1 Comparison of the means of intravenous $AUC_{0\to\infty}$ calculated by computer programme "NONLIN" and by trapezoidal rule.

| | Dose ($mg.kg^{-1}$) | $AUC_{0\to\infty}$ ($\mu g.ml^{-1}.min$) | |
|---|---|---|---|
| | | "NONLIN" | Trapezoidal rule |
| Lignocaine | 2.5 | 188.0 | 174.8 |
| | 5.0 | 444.7 | 441.8 |
| | 10.0 | 758.4 | 753.8 |
| Tocainide | 5.0 | 571.8 | 565.2 |
| | 20.0 | 2293.4 | 2290.0 |
| | 35.0 | 4001.5 | 3987.9 |
| | 50.0 | 5901.9 | 5896.1 |

A bi-exponential decline in blood lignocaine concentration after intravenous administration has also been found in monkey (Benowitz et al, 1974 a and b) and man (Boyes et al, 1971; Rowland et al, 1971; Thomson et al, 1973; Perucca and Richens; 1979). The initial ($\alpha$) exponential decay phase in rat, with an average half-life of 6.5 minutes, is similar to that of 5 to 10 minutes reported in man by Boyes et al (1971); Rowland et al (1971); Perucca and Richens (1979) and to that of 1.5 minutes described in monkey by Benowitz et al (1974a). The $t_{\frac{1}{2}}$ of the $\beta$ phase of decline in blood concentration in rat (52 minutes) is comparable to that found in dog (40 minutes) reported by Boyes et al (1970), monkey (15 minutes) and man (80 to 108 minutes). The Vp (approximately 0.56 $l.kg^{-1}$) and $Vd_{ss}$ (0.93 $l.kg^{-1}$) were similar to those found in dog, monkey and man.

Rapid equilibration of intravenously administered lignocaine between blood and highly perfused organs is also indicated by other techniques. Katz (1968) and Keenaghan and Boyes (1972) using radioactive labelled lignocaine found that immediately after injection 70% of the activity

was found in vessel-rich tissues (heart, lung, liver, brain, kidney and spleen). The rapid distribution of lignocaine was also reported in monkey by Benowitz et al (1974a) using a perfusion model. They estimated that within 0.5 minute after injection, 70% of drug had left the blood pool and had entered lung, viscera, and muscle. It is also noticeable that the peak concentrations of lignocaine after intravenous injection depend on the speed of administration. In the present experiments when injection time was reduced to less than 30 sec, rats which received the highest dose of lignocaine (10 $mg.kg^{-1}$) died within seconds of injection presumably because of high concentration in essential organs. Significantly the most common cause for toxicity of lignocaine in patients was reported by Collinsworth (1976) to be excessivley rapid administration of the drug. Akerman et al (1966) found that the concentration of lignocaine in numerous organs of rat (brain, liver, lung, kidney, heart and spleen) to be higher than those in blood 2 hours after intramuscular injection at the dose of 10 $mg.kg^{-1}$ indicating considerable localisation of the drug in tissues.

Blood concentration data for lignocaine following oral administration in man have been analysed by computer fitting by Boyes et al, (1971), using Vp, $k_{12}$, $k_{21}$ and $k_e$ calculated from the intravenous data and allowing $k_{ab}$ to vary. In the present work, this technique was not used. The blood concentration data of lignocaine after single oral administration, therefore, were fitted by least squares linear regression analysis. The $t_{\frac{1}{2}}$ of lignocaine after oral administration was similar to that found after the drug had been given intravenously and peak concentrations were acheived within 30-45 minutes after drug administration. The findings are compatible with rapid absorption of lignocaine as has been found in man (Boyes et al, 1971; Perucca and Richens, 1979) and in dog (Boyes et al, 1970).

The AUC for lignocaine after oral administration, however, was considerably less than the AUC after intravenous administration when comparable doses are considered indicating extensive presystemic elimination of the drug. Indeed the area ratio gave a mean value for availability (F) of 0.019, that is an hepatic extraction (E) of 0.981. The value for hepatic extraction in the present study agrees well with those of Pang and Rowland (1977b) who used the perfused rat liver in situ and obtained a value for E of 0.99. Indeed hepatic extraction of lignocaine in rat is highest of all species studied, values for E in other animals being dog 0.78 (Branch et al, 1973), man 0.7 (Boyes et al, 1970; Perucca and Richens, 1979; Bennett et al in press), monkey 0.66 (Benowitz et al, 1974 a) and cat 0.28 (Lautt and Skelton, 1976).

In rat and in dog recovery of radioactively labelled drug and metabolites in urine after oral and intravenous administration of lignocaine are similar (Keenaghan and Boyes, 1971), indicating that absorption of lignocaine from the gut is complete as well as rapid. Pre-systemic elimination of an orally administered drug may be due to metabolism either in the gut wall, in the liver or in the lung. There is considerable evidence that the major site of elimination of lignocaine is the liver (Sung and Traunt, 1954; Beckett et al, 1966; Boyes et al, 1971). In most species studied the hepatic clearance of lignocaine is high for example in monkey (269 $ml.min^{-1}$; Benowitz et al, 1974a); dog (433 $ml.min^{-1}$; Branch et al, 1973) and man (0.54-1.54 $l.min^{-1}$; Stenson et al, 1971). The values approximate to 1 $ml.min^{-1}.gm$ of liver that is, a figure approaching that for liver blood flow. The present findings in rat are well in agreement with those for other animals. Thus although there are differences in the pattern of metabolites (Keenaghan and Boyes, 1972) the rat appears to be comparable to the other species

studied in that it is capable of extensive extraction of lignocaine by the liver from the blood. Thus as in other species lignocaine may be be regarded in the rat as a blood flow dependent drug and changes in hepatic blood flow will be reflected in changes in the elimination of lignocaine.

In the present work the kinetics of lignocaine after intravenous administration did not change with dose, the blood concentration-time curves were superimposable and AUC increased linearly with dose, the regression line of the plot of AUC against dose passing through the origin. There is strong evidence therefore, that the kinetics of lignocaine are linear over the dose range tested, i.e. the metabolic and disposition processes were not saturated. Studies of this type in the literature are scarce. Bennett et al (in press) studying lignocaine kinetics in man found that in 3 normal volunteers AUC increased linearly with doses of 50 and 100 mg, i.v. However, Lalka et al (1976a) who gave constant rate intravenous infusions of lignocaine (40, 80, 120 mg) found that clearance decreased with increase in dose in man suggesting that the kinetics were dose-dependent under these conditions. Furthermore, in a study by Bennett et al (in press) following multiple oral dosing with lignocaine 300 mg eight hourly for 7 doses, one subject appeared to show saturable kinetics whereas no evidence of saturation was found in two other subjects. The issue of time-dependency was not explored in the present work with lignocaine because it was planned only to use single doses in later work.

After oral administration no change was found in the absorption rate constant ($k_{ab}$) or in the disposition rate constant ($k_d$) with change in dose. AUC increased linearly with dose in the range 50-400 $mg.kg^{-1}$ but it was noted that the regression line relating AUC to dose did not pass through the origin, rather it crossed the dose axis at a point

equivalent to a dose of 23 $mg.kg^{-1}$. In an attempt to define the relationship at lower levels doses of 10 $mg.kg^{-1}$ and 30 $mg.kg^{-1}$ were given orally to rats but the blood lignocaine concentrations acheived in these experiments were too often below the acceptable limit of sensitivity of the assay and the AUC could not adequately be defined. Shand and Rangno (1972) made a similar finding in human subjects who took propranolol by mouth in that the line relating AUC to dose crossed the dose axis at a dose of 30 mg. They proposed a separate extraction process for propranolol which at low doses avidly extracts the drug from plasma. It is possible that a similar process may operate for lignocaine in the rat but this was not studied further in the present work. Furthermore, since the point at which the trend line crosses the dose axis is included in the 95% confidence interval for the line it may not even be necessary to evoke a separate mechanism for the elimination of lignocaine at low doses.

Systemic availability of orally administered lignocaine did not change within the dose range studied (Table 3.5) and similar conclusions were made by Bennett et al (in press). Earlier studies by Boyes et al, (1971) have suggested that in some subjects oral availability of lignocaine may increase with dose.

The present studies in summary have shown that in the rat there is a dose range over which the kinetics of lignocaine are clearly linear and it is therefore possible to select a dose for further studies in which factors influencing the elimination of lignocaine may be conducted. Furthermore, as in other species, lignocaine is obviously a drug which is highly cleared by the liver and it can thus be regarded as blood flow dependent for its elimination. Thus the rat has been shown in the present work to be a suitable experimental animal in which to conduct studies of lignocaine kinetics and the data obtained are comparable to those previously established for other species such as dog, monkey and man.

## 6.3 Basic kinetics of tocainide in the rat

Tocainide is a structural anaogue of lignocaine and appears to possess many of the pharmacodynamic properties of the parent drug (Winkle et al, 1978). In contrast the pharmacokinetic properties of tocainide are very different from those of lignocaine.

In the present study the GLC technique for assaying tocainide in the rat was sufficient to provide accurate estimations to concentrations of 0.1 $\mu g.ml^{-1}$ which was sufficinet to define the blood concentration-time curves within the dose-range selected. No apparent loss occurred in storage of the samples at $-20^{o}C$ for up to 3 months.

Most available kinetic data on tocainide have been obtained in man and the present work is believed to be the first full analysis of the kinetic properties of the drug in an animal species. The blood concentration-time data after intravenous administration were analysed by the non-linear least squares regression analysis programme NONLIN. (Metzler, 1969). It was observed that after intravenous administration of tocainide to rats the blood concentration-time curve exhibited a biexponential form with a relatively short initial phase (average half-life of 9.2 minutes) and a much longer secondary phase (average half-life of 127.7 minutes). As for lignocaine, the data could be adequately described by a two-compartment open system. The $t_{\frac{1}{2}}$ of the $\beta$-phase of elimination of tocainide from the blood is substantially longer than that of lignocaine ($t_{\frac{1}{2}\ \beta} \simeq 52$ minutes). A similar difference is also found in man which $t_{\frac{1}{2}\ \beta}$ for tocainide is 11 hours (Lalka et al, 1976) in contrast to that of lignocaine (100 minutes). The more gradual elimination of tocainide than lignocaine from the blood is one reason why it has become used for the long term management of arrhythmias in man.

The volume distribution constants (Vd) were all of the order of 1-2 $l.kg^{-1}$ implying that tocainide like lignocaine undergoes considerable extravascular distribution. These data are similar to the findings in man (Lalka et al, 1976; Graffner et al, 1979).

After oral administration peak blood concentration of tocainide were acheived within 2-4 hours. The logarithm of blood concentration during the disposition phase was linearly related to time and $t_{\frac{1}{2}}$ at doses of 100, 200, 300 and 500 $mg.kg^{-1}$ ranged between 8.3-11.7 hours but are not significantly different from each other. However, $t_{\frac{1}{2}}$ of the lowest dose (50 $mg.kg^{-1}$) was significantly shorter at 4.6 hours than that for the other doses. Doses below 50 $mg.kg^{-1}$ were not explored because blood concentration attained could not be measured with sufficient accuracy to define the concentration-time curves. The significant feature when comparing $t_{\frac{1}{2}}$ after i.v. and after p.o. administration of tocainide was the much longer $t_{\frac{1}{2}}$ by the latter route. Absence of drug from faeces and gut contents after a single oral dose of tocainide to rate (3.6) suggest complete absorption of the drug from the alimentary tract, as does the similarity of AUC obtained after p.o. and i.p. administration of 50 $mg.kg^{-1}$ (Table 3.11). The longer $t_{\frac{1}{2}}$ of tocainide after oral administration thus suggests that although a substantial amount of the drug may be absorbed rapidly to give the early peak concentrations absorption, thereafter continues for several hours. Because both absoprtion and disposition are first order processes the logarithm of drug concentrationin the disposition phase is still linearly related to time. The half-life of tocainide (50 $mg.kg^{-1}$) after i.p. injection was significantly shorter than that after p.o. administration ($p<0.001$) but significantly longer than that after i.v. injection ($p<0.001$). This suggests that although diffusion of tocainide from the peritoneal cavity is more rapid than absorption from the gut, even from the peritoneal

cavity there exists a significant barrier to diffusion. Although there are no human studies in which $t_{\frac{1}{2}}$ of tocainide was measured after i.v. and p.o. administration to the same subjects Lalka et al (1976), found that $t_{\frac{1}{2}}$ after intravenous infusions was 11 hours whereas McDevitt et al (1976) found that the value after p.o. administration was 14 hours, suggesting that in man slow absorption from the gut may also contribute to the finding of a longer $t_{\frac{1}{2}}$ when the drug is given by the oral route. On the other hand these differences in man could simply be due to sampling error and a proper comparison of $t_{\frac{1}{2}}$ after i.v. and p.o. administration is needed to clarify the issue.

Renal clearance of tocainide was on average 20.5% and remained relatively constant when dose was changed (range 17.9-22.4%). These values are approximately half those found in man by Lalka et al (1976). In the latter study it was found that urinary clearance of tocainide could be reduced by alkalinisation but urine pH was not controlled in the present work. It has been assumed that renal clearance of tocainide is the same irrespective of the route of administration. Thus estimates of AUC still give a value for availability. Calculation of F for doses of 100, 200, 300 and 400 $mg.kg^{-1}$ in relation to the mean of all intravenous doses gave an average value for availability of 0.94 that is an hepatic extraction (E) of 0.06.

The constancy of the kinetic parameters after intravenous and after oral dosing in the range 100-400 $mg.kg^{-1}$, the superimposibility of dose-normalised blood concentration-time curves and the linear relationship between AUC and dose provide good evidence for regarding the kinetics as showing dose-independency over the range studied.

Because tocainide is a relatively new drug and is proposed for long term use in man the studies of tocainide were extended to include multiple doses. At a dose selected from the middle of the linear

range for single intravenous dose studies (20 $mg.kg^{-1}$), five of six rats showed no change in $t_{\frac{1}{2}}$ or AUC after 7 doses when it was assumed that steady state would have been attaned (4 x $t_{\frac{1}{2}}$) indicating that the kinetics were substantially time-independent under these conditions. For multiple oral dosing a dose of which was higher (200 $mg.kg^{-1}$) but which was still within the linear range for the single oral dose studies was selected. The quality of the data was less satisfactory but three out of five animals showed no change in $t_{\frac{1}{2}}$ or AUC after 12 doses whereas two rats showed altered kinetics ($t_{\frac{1}{2}}$ or AUC) and evidently suffered from drug toxicity. On a weight related basis these oral doses are substantially in excess of the dosing regimens used in man.

The kinetics of tocainide in rat thus shows dose-independence and in a proportion of animals time-independence under the stated conditions. Although a detailed exploration of the kinetics of tocainide has not been conducted in another species, Lalka et al (1976) in man found that for oral doses between 50 mg and 850 mg, AUC increased linearly with dose indicating stable kinetics for this dose range.

Hence, tocainide, a drug structurally and pharmacodynamically similar to lignocaine has been shown in the rat to be pharmacokinetically very different from lignocaine. Although lignocaine relies almost exclusively (99%) and tocainide very largely (80%) on the liver for elimination, the hepatic extraction of tocainide (0.06) contrasts sharply with that for lignocaine (0.99). Consequently alteration in liver blood flow would be anticipated to have little influence on the elimination of tocainide but changes in drug metabolism would be expected to alter the kinetics of tocainide significantly. The rat has therefore been shown to be a satisfactory experimental animal in which to examine the influence of such variables on the elimination of tocainide.

## 6.4 Effect of enzyme induction on the kinetics of lignocaine and of tocainide in rat

### 6.4.1 Enzyme Induction

The duration and intensity of action of many drugs are largely determined by the speed with which they are metabolised by enzymes in liver microsomes. The activites of drug metabolising enzymes in liver microsomes can be markedly increased when animals are treated with various hormones, drugs, insecticides or carcinogens. This increase in activity appears to represent an increased concentration of enzyme protein and is referred to as "enzyme induction" (Conney, Davison, Gastel and Burns, 1960; Conney, 1967; Remner, 1972). The induction of liver microsomal enzymes is important pharmacologically, for it leads to an accelerated biotransformation of drugs and so alters duration and intensity of drug action. It is evident that enzyme induction is likely to have most effect on the kinetics of drugs which exhibit low hepatic clearance i.e. drugs for which the rate of metabolism governs elimination. Kinetics of flow-dependent drugs might be expected to be less affected. However, the picture is further complicated by the fact that some enzyme inducing drugs also alter blood flow. This section of the work is concerned with the effect of altering metabolism by enzyme induction on the kinetics of lignocaine (blood flow dependent) and of tocainide (metabolism dependent). There is a particular practical relevence to understanding the effect of enzyme induction on the kinetics of tocainide, since the latter drug is proposed for long term oral use in patients for whom maintenance of therapeutic blood concentrations is important. Because of the variable effects on blood flow of known enzyme inducers two agents were selected; 3,4 benzpyrene which is a potent enzyme inducer without effect on liver blood flow

and phenobarbitone which has been shown in rat to induce hepatic enzymes and to increase liver blood flow. (Ohnhaus et al, 1971;Ohnhaus, et al, 1975). Phenobarbitone is known to stimulate various drug metabolic reactions including oxidation, reduction, de-esterification and glucuronide formation. In contrast 3,4 benzpyrene induces a more limited group of reactions (Conney, Davison, Gastel and Burns, 1960; Conney, Gillette, Inscoe, Trams and Posner, 1963; Conney, 1967). The time course by which enzyme induction is increased has been well studied and is different for the two drugs. After daily administration of phenobarbitone to rats the maximal increase of enzyme activity is acheived after least 3 days (Conney et al, 1960; Ohnhaus et al, 1971) whereas after a single intraperitoneal injection of 3,4 benzpyrene enzyme activity more than doubles within 3-6 hours, the maximal increase is observed within 24 hours and the effect lasts for many days (Conney et al, 1957; Ohnhaus et al, 1971). Because these time relationships have been well studied in the past, they were not repeated in the present work. However, the essential changes brought about by the administration of 3,4 benzpyrene and of phenobarbitone are given (Chapter 4.1). These showed that pentobarbitone sleeping time was reduced (Table 4.1) indicating increased metabolism and reduced biological effect of that drug. An enzyme inducing dose of 3,4 benzpyrene had no effect on liver weight but administration of phenobarbitone caused a 26% increase in liver weight (Table 4.2) and this finding has also been made by other workers (Ohnhaus et al, 1971; Ohnhaus and Locher, 1975; Nies et al, 1976). Both enzyme inducers caused an increase in microsomal protein and in cytochrome $P_{450}$ content (Table 4.3) confirming well established previous work (Orrenius and Ernster, 1964; Ohnhaus et al, 1971; Remmer, 1972). It was noted that increases in microsomal protein and cytochrome $P_{450}$ expressed per gram of liver were not different in

phenobarbitone and in 3,4 benzpyrene treated rats but since phenobarbitone administration was associated with an increased liver weight whereas 3,4 benzpyrene was not, there was a greater total increase in microsomal protein and cytochrome $P_{450}$ assocated with phenobarbitone administration. Enzyme kinetics were performed using the activity of microsomal O-demethylation of p-nitroanisole as an indicator of overall liver microsomal metabolising activity although this particular enzyme is not involved in the biotransformation of lignocaine or of tocainide. Administration of phenobarbitone and 3,4 benzpyrene were associated with increased $V_{max}$ whereas $K_m$ was unaltered indicating an increase in enzyme activity without alteration substrate affinity (Table 4.4).

### 6.4.2 Enzyme induction and haemodynamic change

Since this work is concerned with the differing effects of drug metabolism and blood flow on drug kinetics the influence of 3,4 benzpyrene and of phenobarbitone on regional blood flow and in particular on hepatic blood flow were investigated (Chapter 4.2). It was also necessary to establish whether lignocaine or tocainide independently altered any haemodynamic parameters and the data for these drugs is conveniently considered at this point. The parameters measured were; mean arterial blood pressure, cardiac output and organ blood flow. Changes in the weights of individual organs were also noted.

No change in mean arterial pressure or in cardiac output were found after administration of phenobarbitone, 3,4 benzpyrene, lignocaine or tocainide. Likewise, none of these drugs produced change in blood flow to or weight of the heart, lungs or kidneys. When liver blood flow and liver weight are considered no change was found after administration of 3,4 benzpyrene, lignocaine or tocainide. However, administration of phenobarbitone was associated with a significant

increase in hepatosplanchnic (total liver) blood flow. With the radioactively labelled microsphere technique the two components of blood flow to the liver, namely hepatic arterial and portal flow are measured separately. It was found that the increase in hepatosplanchnic flow was entirely due to increased portal blood flow and in particular that portion of portal flow which came from the GI tract and pancreas, there being no increase in splenic blood flow. Because this increase in liver blood flow after phenobarbitone was associated with an increase in liver weight, the hepatic blood flow per gram of liver was not increased. Other workers who have used this technique have made the same findings in respect of changes in organ and in particular liver blood flow after 3,4 benzpyrene and phenobarbitone (Ohnhaus et al, 1971; Nies et al, 1976). The absence of haemodynamic change after administration of lignocaine or of tocainide to rats is not unexpected and accords with the general findings of other workers using different techniques and different species (Chapter I). Benowitz et al (1974a), however, using the labelled microsphere technique found that infusion of lignocaine did cause changes in the distribution of cardiac output, the fraction going to hepatic artery, long bone and heart increasing at the expense of skeletal muscle. The present observations in rat contrast with these findings. Studies of the influence of tocainide on organ blood flow using the labelled microsphere technique have not been reported previously.

It is appreciated that a full analysis of the drug studied in this section would also include the effect on haemodynamics of the combinations of drugs used. Since no change in haemodynamic parameters was observed after 3,4 benzpyrene, lignocaine or tocainide, such a study was not undertaken for these drugs. However, because changes were observed after phenobarbitone, the effect of the combination of phenobarbitone followed by tocainide was studied and the same findings were made as with phenobarbitone alone.

6.4.3 Enzyme induction and pharmacokinetic change

It is apparent from Chapter 4 that enzyme induction brought about changes in the kinetics of lignocaine and tocainide. In this section these changes are considered as observation and their consequences are discussed. In a later section the change will be considered in relation to the prediction of pharmacokinetic models.

Intravenous administration

Lignocaine is a drug which undergoes high hepatic clearance and it would be anticipated that change in drug metabolism would have relatively less effect than change in blood flow especially when the drug is given intravenously. The observations support this in that change in metabolism alone (after 3,4 benzpyrene) caused no change in $t_{\frac{1}{2}}$, AUC or CL. When phenobarbitone was given, however, AUC was signficiantly reduced and CL increased but there was no change in $t_{\frac{1}{2}}$. The changes after phenobarbitone presumably reflect the additional effect of change in blood flow caused by this drug but they could also be due to a more intensive enzyme inducing effect.

Tocainide undergoes low hepatic extraction and consequently change in drug metabolism would be expected to have more marked effects on its kinetic than change in blood flow. Thus any effect observed after administration of phenobarbitone can presumably be ascribed to change in drug metabolism and not to change in liver blood flow. In effect both 3,4 benzpyrene and phenobarbitone caused AUC of tocainide to decrease and CL increased after intravenous administration. The changes were more marked after phenobarbitone and $t_{\frac{1}{2}}$ was also reduced with this drug. If the effect of increased blood flow may be ignored, these changes presumably reflect a greater effect on the metabolism of tocainide when the rats were pretreated with phenobarbitone.

Oral administration

When a drug is given by the oral route it must all pass through the liver once in order to reach the systemic circulation. This contrasts with intravenous administration for which in any single pass through the liver only drug in that part of cardiac output which supplies the liver (about 25%) is available for metabolism. For drugs which are highly cleared by the liver this difference in the fraction which is exposed to hepatic degradation depending on the route of administration is important and is expressed as extensive pre-systemic elimination by the oral route (first pass effect). With such drugs when factors governing elimination are altered the effects on kinetics are likely to be more marked when the drug is given by the oral route. The findings after oral administration of lignocaine to 3,4 benzpyrene treated rats were that although there was no change in $t_{\frac{1}{2}}$ there was a significant reduction in AUC. After phenobarbitone there was an even greater reduction in AUC.

Significant changes were also observed after oral administration of tocainide in that with the enzyme inducers, both $t_{\frac{1}{2}}$ and AUC were reduced, the effect on AUC being more marked with phenobarbitone. This may reflect a more potent effect in increasing drug metabolism with the latter drug.

Comparison with AUC after p.o. and i.v. administration allows calculation of a value for availability (F). For a highly extracted drug such as lignocaine changes in availability have relatively little practical consequence but in fact after both enzyme inducers F fell, the mean values being control 0.019, 3,4 benzpyrene 0.013 and phenobarbitone 0.010. Tocainide is much less extracted in a single pass through the liver than lignocaine. Extraction of tocainide was also increased with reduced availability, the mean values for F being control 0.94, 3,4 benzpyrene 0.64 and phenobarbitone 0.63.

Possible consequences in clinical use

The findings may be extrapolated to the clinical use of these drugs. They imply that for lignocaine, a drug used almost exclusively by the intravenous route, since $t_{\frac{1}{2}}$ does not change, the time to reach steady state (4 x $t_{\frac{1}{2}}$) would be unaltered by enzyme induction. CL was increased but not significantly so by 3,4 benzpyrene but was significantly increased by phenobarbitone. Thus following intravenous infusion it is possible that the concentration of lignocaine at steady state may be reduced in enzyme induced patients who would thus not receive the degree of protection expected from standard dosing regimens. (Perucca and Richens, 1979), although reduction in concentration of lignocaine would be mitigated by the fact that the primary metabolite MEGX is also pharmacologically active.

Tocainide may also be given by the intravenous route. The present data indicates that in the presence of enzyme induction CL is increased and thus the steady state blood concentration anticipated from standard dosing regimens would be reduced. The data for phenobarbitone (reduced $t_{\frac{1}{2}}$) also suggest that steady state may be attained in a shorter time in enzyme induced subjects. The major use of tocainide, however, is oral and the data clearly show that although time to attain steady state is reduced (reduced $t_{\frac{1}{2}}$), the major reduction is in AUC and is indicative of reduced blood concentration in the presence of enzyme induction. In patients on standard dosing lower than expected steady state concentrations would be acheived and such individuals may not be adequately protected against the risk of cardiac arrhythmia.

## 6.5 Effects of reduction in blood flow on the kinetics of lignocaine and tocainide in rat

### 6.5.1 Liver blood flow

The various methods which have been used to measure liver blood flow are reviewed in Chapter 1. In the present study the radioactively labelled microsphere method was used because it not only gave reliable data for liver blood flow in rat but flow to other organs and the fractional distribution of cardiac output can also be measured. The values obtained for flow to liver and other major organs in the present study are in good agreement with those of McDevitt and Nies (1976), Hiley and Yates (1978). Liver blood flow relative to tissue weight varies little between species being of the order of 1 $ml.min^{-1}.g_{tiss}^{-1}$ and the findings in the present work are in accord with this (Neutze, Wyler and Rudolph, 1968; Sasaki and Wagner, 1971; Benowitz et al, 1974a; Yates et al, 1979).

Liver blood flow is an important determinant of the elimination of many drugs, particularly those with high extraction ratio. Change in cardiac output or in distribution of the output to the liver cause variation in blood flow to this organ and may thus affect drug clearance. Factors that generally regulate cardiac output and its distribution have been summarized by Wilkinson (1975). Exercise and thermal stress which increase cardiac output, caused about a 50% increase in human hepatic elimination of indocyanine green, a highly hepatic cleared substance (Rowell, Blackmen, Martin, Mazzarella and Bruce, 1965), whereas the hepatic clearance of antipyrine, a drug with a low hepatic extraction ratio, was unaffected by these stresses (Schartz, Sidell and Cucinell, 1974). Other physiological variations e.g. changes in posture from the supine to upright position which decrease cardiac output, which leads to a significant reduction in flow to the liver were reported by

Culbertson, Wilkins, Ingelfinger and Bradley (1951) to produce a two-fold difference in the metabolism of aldosterone. Liver and heart disease may alter cardiac output and/or blood distribution leading to changes in hepatic blood flow. Thomson et al (1971) reported that there was about a 55% decrease in lignocaine clearance in patients with cardiac failure compared with normal. Other factors that cause haemodynamic alterations are drugs. The clearance of d-propranolol was increased in the presence of glucagon in the monkey (Branch et al, 1973) because glucagon produced a dose-related increase in cardiac output and in total liver blood flow.

In the restrained monkey Benowitz et al (1974b) found that infusion of isoprenaline lowered and infusion of noradrenaline raised steady state blood concentration of lignocaine due to alteration of liver blood flow. Branch et al (1974) demonstrated that phenobarbitone pretreatment caused an increase in the clearance of d-propranolol with reduction in $t_{\frac{1}{2}}$. They estimated that only 43% of the increase in clearance was due to enzyme induction and that 57% was due to the fact, also found in the present work, that phenobarbitone increases liver blood flow. The same study also indicated that change in liver blood flow only played a minor role in the elimination of antipyrine a drug for which $E \simeq 0.2$.

Branch et al (1973) demonstrated that when the pharmacologically active dl-propranolol was given, cardiac output and liver blood flow were reduced and that the half life longer and clearance of lignocaine were reduced. d-propranolol the pharmacologically inactive isomer caused no change in the kinetics of lignocaine indicating that the alteration in the kinetics were due to the effects of β-adrenoceptor blockade on haemodynamics. In similar studies with oxyphenbutazone a drug with low hepatic extraction the kinetic effects of β-adrenoceptor

blockade were predictably less. The isomers of propranolol provide further evidence of effect of liver blood flow on drug clearance. Nies et al (1973) found that in the monkey the clearance of dl-propranolol was about 25% less than that of d-propranolol an effect which may be attributed to the β-adrenoceptor antagonistic effect of the l-isomer.

### 6.5.2 β-adrenoceptor blockade and organ blood flow

The labelled microsphere method permits an analysis of the blood flow to individual organ and also the fractional distribution of cardiac output. β-adrenoceptor blockade with sotalol in the present experiments brought about the expected decrease in cardiac output (26 %, $p<0.02$). This decrease was reflected in reduced blood flow received by all organs but was only statistically significant in the case of hepatic arterial flow. Blood flow to alimentary tract, pancreas and spleen were also reduced but not to a statistically significant degree. However, when hepatosplanchnic (hepatic arterial plus portal) flow was considered, blood flow was reduced by 29% which was statistically significant ($p<0.05$). Because the reduction in cardiac output was reflected in reduced blood flow to all organs the regional distribution of flow was not significantly altered by treatment with sotalol.

The effects of pentobarbitone anaesthesia per se was studied in dogs by Kaihara, van Heerden, Migita and Wagner (1968) in rats by Bell, Hiley and Yates (1977), using the microsphere distribution technique and in man by Goldberg (1970) using the Fick's method. All studies found that pentobarbitone did not affect splanchnic haemodynamics.

6.5.3 Beta-adrenoceptor blockade and pharmacokinetic change

The present study has been concerned with lignocaine which is liver blood flow dependent for elimination and tocainide one which is not flow dependent. Thus it would be expected that altering liver blood flow would have different effects on the kinetics of these two drugs.

Blood flow was altered by administering a β-adrenoceptor antagonist, since this is a convenient way to alter flow and one which is likely to be encountered in use in man. In the selection of a β-blocking agent it was necessary to find a drug which gave a depression in blood flow for a sufficient length of time to permit change in the kinetics of the two drugs to be observed. It was thus necessary to have a drug with a reasonaly long $t_{\frac{1}{2}}$. Sotalol was selected because it has $t_{\frac{1}{2}}$ of $\simeq$ 3 hours after intraperitoneal injection of 20 $mg.kg^{-1}$ in the rat (Ishizaki, Tawara and Oriuchi, 1977). Sotalol is also eliminated mainly by the kidney which is an added advantage because there is less chance of interaction between sotalol and lignocaine or tocainide both of which are eliminated by the liver.

The data may be dealt with either on observations or in relation to the changes in kinetics predicted by the pharmacokinetic models. In this section the data will be dealt with as observations and the implications of the findings on dosing are discussed.

In initial experiments it was found that doses up to 50 $mg.kg^{-1}$ did not have a sufficiently prolonged action for the proposed experiments in the rat. Doses in excess of 100 $mg.kg^{-1}$ was lethal. Thus a dose of 100 $mg.kg^{-1}$ was selected and a regime in which sotalol was given intraperitoneally three times on the day before the experiment, then again 15 minutes before lignocaine or 30 minutes before tocainide and in case of tocainide on three further occassions was found to be satisfactory (Chapter 5.1). As was anticipated, when sotalol pre-

treated rats were given lignocaine changes in kinetics were observed; after intravenous administration both $t_{\frac{1}{2}}$ and AUC were increased and inconsequence drug clearance was reduced. Similar change occurred after oral administration with lengthening of $t_{\frac{1}{2}}$ and increase in AUC.

The findings after tocainide were those that would be expected of a drug which has low hepatic extraction. There was a slight increase in AUC and lengthening of $t_{\frac{1}{2}}$ after intravenous tocainide but the changes were not significant statistically. Increase in AUC and $t_{\frac{1}{2}}$ also occurred after oral administration of tocainide but again the changes were not significant. It may be recalled that 20% of a dose of tocainide is eliminated by the kidney but this may be ignored because renal blood flow, showed only minor changes.

Possible consequences inclinical use

The finding can be extrapolated to man. It is possible that in patients who are fully β-blocked the haemodynamic and kinetic changes will be the similar as those made in the present work, i.e. lengthened lignocaine $t_{\frac{1}{2}}$ and reduced clearance. This would imply that such patients receiving constant rate intravenous infusions of lignocaine would attain steady state after a longer time than would non-β-blocked patients and because clearance is reduced, the ultimate steady state concentration attained would be higher than in non-β-blocked patients. Thus unless the dosage scheme was modified patients receiving βadrenoceptor blocking drugs would run increased risk of toxicity from lignocaine infusion.

The findings are also of value for tocainide because they imply that even patients who are β-blocked would not show altered kinetics. Thus normal dosing schedules for tocainide probably apply to patients taking β-blockers.

## 6.6 Liver blood flow: comparison of results obtained by the microsphere and by the lignocaine clearance methods

Two methods were used in this work to measure liver blood flow. This provides an opportunity to compare the results obtained with the lignocaine clearance and with the radioactively labelled microsphere techniques when liver blood flow is altered by the experimental conditions. Mean data for control and for 3,4 benzpyrene, phenobarbitone and sotalol pretreated rats are summarised in Table 6.2.

The values for hepatic blood flow in control animals obtained by the two techniques were different being approximately three times greater by the microsphere method. The reason for this difference is not clear. The data for the microsphere method agrees with previously published work by McDevitt and Nies (1976) and Hiley and Yates (1978) but data for liver blood flow in rat using the lignocaine clearance technique have not been found in the literature. Anaesthesia (ether + pentobarbitone) may have affected the results with the microsphere technique but this form of anaethesia is reported not to affect liver blood flow (Bell et al, 1977). Table 6.2 also shows that there was agreement between the two methods in the direction of change in liver blood flow under the influence of the various drugs used but the magnitude of the change was different being approximately two times greater when liver blood flow was calclulated by the lignocaine clearance techniqe. Thus the increase in liver blood flow after phenobarbitone was 39.7% by the microsphere method and 76.7% by the lignocaine clearance technique. The fall in liver blood flow after sotalol was 22.7% by the microsphere method but 50.9% by the lignocaine clearance technique.

The two methods differ in approach to the estimation of liver blood flow, one depending on the clearance of a drug, the other on the arrest of microspheres in the hepatic microvasculature. The quantitative differences in the data obtained by the two techniques suggest that there are differences in what the two methods measure but the explanation for this is obscure.

Table 6.2 Comparison of mean liver blood flow calculated by the microsphere and by the lignocaine clearance methods in control and in treated rats

| Treatment | liver blood flow | | | | | |
|---|---|---|---|---|---|---|
| | by lignocaine clearance | | | by microspheres | | |
| | $ml.min^{-1}$ | $ml.min^{-1}.100g,bw^{-1}$ | % change | $ml.min^{-1}$ | $ml.min^{-1}.100g,bw^{-1}$ | %change |
| control | 5.28 | 1.26 | — | 20.74 | 4.06 | — |
| 3,4 benzpyrene | 6.64 | 1.47 | (+16.5) | 17.9 | 4.16 | (+2.5) |
| phenobarbitone | 9.58 | 2.23 | (+76.7) | 27.13 | 5.67 | (+39.7) |
| sotalol | 2.45 | 0.62 | (-50.9) | 11.14 | 3.14 | (-22.7) |

## 6.7 Observed values compared with predictions of Model I and Model II

The observed values for various pharmacokinetic parameters derived in this work were compared with those predicted by Model I and Model II.

### Change in liver blood flow

The definition of intrinsic clearance is the same with both models but the predicted value derived from a given extraction ratio does differ. Intrinsic clearance can be derived from each model from

Table 1.2 in terms of blood flow and extraction as follows:

Model I $CL_{int} = \frac{QE}{1-E}$

Model II $CL_{int} = -Q \cdot \ln (1-E)$

Using the value for E for lignocaine obtained from the mean of all intravenous and oral single dose studies (0.981), $CL_{int}$ was calculated for each model. Taking the values for liver blood flow (corrected to 100 g, body weight) obtained by the lignocaine clearance and by the microsphere methods predicted values for E, $AUC_{i.v.}$, F and $AUC_{p.o.}$ were calculated for each model.

The predictions for the kinetics of lignocaine in control rats were compared with the findings for rats which had received sotalol. Since $CL_{int}$ is independent of flow the value for $CL_{int}$ calculated for the control rats was used for predicting parameters for sotalol treated rats. The values for liver blood flow after treatment with sotalol (Table 6.3) were substituted to predict the kinetics of lignocaine in the sotalol treated rats. The data for lignocaine appears in Table 6.3. This shows that E did not change when flow was reduced and this is accurately predicted by both models. $AUC_{i.v.}$ increased after sotalol treatment and Model I gave a closer prediction of this change than did Model II. Availability (F) was observed to reduce only marginally in the present work but both models predicted a much greater degree of fall. A marked discrepancy between the predictions of both models and the observed data occurs in $AUC_{p.o.}$. This was observed to undergo a two fold increase when liver blood flow diminished but the predictions of Model I are that there would be no change and Model II predicts a reduction in $AUC_{p.o.}$.

The influence of protein binding was ignored in these calculations because lignocaine exhibits non-restrictive binding i.e. $E > f_B$.

Similar calculations were carried out for tocainide. It is recognised that since tocainide exhibits restrictive binding i.e. $f_B > E$, alteration in binding could influence the findings. Since only whole blood was assayed in the present work the fraction bound was assumed to be constant at a value of 0.5 (Lalka et al, 1976). $CL_{int}$ in control animals was calculated from the mean of all intravenous data and was related to the 50 $mg.kg^{-1}$ oral dose which was also the oral dose used in the studies with sotalol. The data are summarised in Table 6.4. This shows that for tocainide E increased by a fractor of 1.24 when blood flow was reduced by sotalol and this was predicted by both models. A slight increase in $AUC_{i.v.}$ was observed after sotalol and was predicted by the models. Likewise the slight fall in F observed after sotalol was predicted. Only slight change was noted in $AUC_{p.o.}$ and the predictions of both models agree with this.

Change in drug metabolism

Predictions in respect of lignocaine for Model I and Model II for E, $AUC_{i.v.}$, F and $AUC_{p.o.}$ were made keeping liver blood flow constant at the value for control rats and substituting the value for E obtained after 3,4 benzpyrene treatment (0.986). The data appears in Table 6.5. This shows that the reductions in $AUC_{i.v.}$ and $AUC_{p.o.}$ which occurred with lignocaine after treatment with 3,4 benzpyrene were predicted by both models. Predictions for tocainide in rats treated with 3,4 benzpyrene were calculated taking the value for E obtained in that study (0.355) and assuming binding to be constant ($f_B$ = 0.5). The data are shown in Table 6.6.

This shows that increase in drug metabolism with 3,4 benzpyrene was associated with an increase in E, a fall in $AUC_{i.v.}$ and a decrease in $AUC_{p.o.}$ all of which changes were predicted by both models.

Change in liver blood flow and in drug metabolism

The data for $AUC_{i.v.}$ and $AUC_{p.o.}$ for lignocaine and tocainide in rats treated with phenobarbitone (i.e. change in liver blood flow and enzyme induction) were calculated as previously described. Values for $CL_{int}$ after phenobarbitone treatment were calculated using the figures for E obtained in that study (lignocaine E = 0.99, tocainide E = 0.369) and in both instances the value for flow obtained in enzyme induced animals was used. The value for flow measured after phenobarbitone treatment by the lignocaine clearance and by the microsphere methods and the above value for $CL_{int}$ were then used to calculate the data for area. The data are given in Table 6.7. This shows that for both $AUC_{i.v.}$ and $AUC_{p.o.}$ the predictions for lignocaine were accurate but the predictions for tocainide were less so.

Table 6.8 Values for E used in calculations

| | Lignocaine | Tocainide |
|---|---|---|
| control | 0.981 | 0.259 |
| sotalol | 0.982 | 0.321 |
| 3,4 benzpyrene | 0.986 | 0.355 |
| phenobarbitone | 0.990 | 0.369 |

Summary of predictions by Model I and Model II

For a low hepatic clearance drug such as tocainide the models predict changes in E, F and AUC with reasonable accuracy when blood flow or metabolism are altered.

For a high hepatic clearance drug such as lignocaine, satisfactory predictions of kinetics change are obtained when hepatic enzymes are induced. When hepatic blood flow is changed the predictions for E and $AUC_{i.v.}$ are reasonably close to the observed changes but neither model could predict the observed changes in $AUC_{p.o.}$ for change in blood flow.

When blood flow and metabolism were changed the predictions for lignocaine were more accurate than the predictions for tocainide.

Table 6.3 Effects of reduction of liver blood flow on lignocaine pharmacokinetics as predicted by Model I and Model II

| | Observed | Model I | | Model II | |
|---|---|---|---|---|---|
| | | by lignocaine clearance | by microspheres | by lignocaine clearance | by microspheres |
| extraction ratio(E) | | | | | |
| control | 0.981 | 0.981 | 0.981 | 0.981 | 0.981 |
| sotalol | 0.982 | 0.991 | 0.985 | 1.000 | 0.994 |
| sotalol/control | 1.00 | 1.01 | 1.00 | 1.02 | 1.01 |
| $AUC_{i.v.}$ ($\mu g.ml^{-1}.min$) | | | | | |
| control | 445 | 405 | 126 | 405 | 126 |
| sotalol | 823 | 814 | 162 | 807 | 160 |
| sotalol/control | 1.85 | 2.01 | 1.29 | 1.99 | 1.28 |
| availability (F) | | | | | |
| control | 0.019 | 0.019 | 0.019 | 0.019 | 0.019 |
| sotalol | 0.018 | 0.009 | 0.015 | 0.0003 | 0.006 |
| sotalol/control | 0.947 | 0.474 | 0.777 | 0.016 | 0.316 |
| $AUC_{p.o.}$ ($\mu g.ml^{-1}.min$) | | | | | |
| control | 103 | 108 | 33 | 108 | 33 |
| sotalol | 206 | 108 | 33 | 3.6 | 13.4 |
| sotalol/control | 1.996 | 1.00 | 1.00 | 0.03 | 0.40 |

Table 6.4 Effect of reduction of liver blood flow on tocainide pharmacokinetics as predicted by Model I and Model II

| | Observed | Model I | | Model II | |
|---|---|---|---|---|---|
| | | by lignocaine clearance | by microspheres | by lignocaine clearance | by microspheres |
| extraction ratio (E) | | | | | |
| control | 0.259 | 0.149 | 0.149 | 0.139 | 0.139 |
| sotalol | 0.321 | 0.262 | 0.184 | 0.263 | 0.176 |
| $sotalol/_{control}$ | 1.24 | 1.76 | 1.24 | 1.39 | 1.27 |
| $AUC_{i.v.}$ ($\mu g.ml^{-1}.min$) | | | | | |
| control | 4002 | 18687 | 5792 | 19942 | 6189 |
| sotalol | 5133 | 21554 | 6044 | 21484 | 6323 |
| $sotalol/_{control}$ | 1.28 | 1.15 | 1.04 | 1.08 | 1.02 |
| availability (F) | | | | | |
| control | 0.741 | 0.851 | 0.851 | 0.861 | 0.861 |
| sotalol | 0.679 | 0.738 | 0.816 | 0.737 | 0.824 |
| $sotalol/_{control}$ | 0.92 | 0.87 | 0.96 | 0.86 | 0.96 |
| $AUC_{p.o.}$ ($\mu g.ml^{-1}.min$) | | | | | |
| control | 4303 | 22727 | 7042 | 24520 | 7610 |
| sotalol | 4981 | 22727 | 7042 | 22627 | 7440 |
| $sotalol/_{control}$ | 1.16 | 1.00 | 1.00 | 0.92 | 0.98 |

Table 6.5 Effect of induction of hepatic enzyme activity on pharmacokinetics of lignocaine as predicted by Model I and Model II

| | Observed | Model I | | Model II | |
|---|---|---|---|---|---|
| | | by lignocaine clearance | by microspheres | by lignocaine clearance | by microsphere |
| extraction ratio (E) | | | | | |
| control | 0.981 | 0.981 | 0.981 | 0.981 | 0.981 |
| 3,4 benzpyrene | 0.986 | 0.986 | 0.986 | 0.986 | 0.986 |
| 3,4 benzpyrene / control | 1.01 | 1.01 | 1.01 | 1.01 | 1.01 |
| $AUC_{i.v.}$ ($\mu g.ml^{-1}.min$) | | | | | |
| control | 445 | 405 | 126 | 405 | 126 |
| 3,4 benzpyrene | 345 | 403 | 125 | 403 | 125 |
| 3,4 benzpyrene / control | 0.78 | 0.99 | 0.99 | 0.99 | 0.99 |
| availability (F) | | | | | |
| control | 0.019 | 0.019 | 0.019 | 0.019 | 0.019 |
| 3,4 benzpyrene | 0.014 | 0.014 | 0.014 | 0.014 | 0.014 |
| 3,4 benzpyrene / control | 0.74 | 0.74 | 0.74 | 0.74 | 0.74 |
| $AUC_{p.o.}$ ($\mu g.ml^{-1}min$) | | | | | |
| control | 103 | 108 | 33 | 108 | 33 |
| 3,4 benzpyrene | 66 | 79 | 25 | 79 | 25 |
| 3,4 benzpyrene / control | 0.64 | 0.73 | 0.73 | 0.73 | 0.73 |

Table 6.6 Effects of induction of hepatic enzyme activity on pharmacokinetics of tocainide as predicted by Model I and Model II

| | Observed | Model I | | Model II | |
|---|---|---|---|---|---|
| | | by lignocaine clearance | by microspheres | by lignocaine clearance | by microspheres |
| extraction ratio (E) | | | | | |
| control | 0.259 | 0.149 | 0.149 | 0.139 | 0.139 |
| 3,4 benzpyrene | 0.355 | 0.216 | 0.216 | 0.197 | 0.197 |
| 3,4 benzpyrene / control | 1.37 | 1.45 | 1.45 | 1.42 | 1.42 |
| $AUC_{i.v.}$ ($\mu g.ml^{-1}.min$) | | | | | |
| control | 4002 | 18687 | 5792 | 19942 | 6189 |
| 3,4 benzpyrene | 2378 | 12864 | 3987 | 14121 | 4379 |
| 3,4 benzpyrene / control | 0.59 | 0.69 | 0.69 | 0.71 | 0.71 |
| availability (F) | | | | | |
| control | 0.741 | 0.851 | 0.851 | 0.861 | 0.861 |
| 3,4 benzpyrene | 0.645 | 0.784 | 0.790 | 0.803 | 0.803 |
| 3,4 benzpyrene / control | 0.871 | 0.921 | 0.923 | 0.932 | 0.933 |
| $AUC_{p.o.}$ ($\mu g.ml^{-1}.min$) | | | | | |
| control | 4303 | 22727 | 7042 | 24520 | 7610 |
| 3,4 benzpyrene | 2190 | 14409 | 4504 | 16204 | 5029 |
| 3,4 benzpyrene / control | 0.51 | 0.63 | 0.64 | 0.70 | 0.66 |

Tabl 6.7 Effects of alteration of hepatic blood flow and enzyme activity on AUC of lignocaine and tocainide as predicted by Model I and Model II

| | observed | Model I | | Model II | |
|---|---|---|---|---|---|
| | | by lignocaine clearance | by microspheres | by lignocaine clearance | by microspheres |
| Lignocaine | | | | | |
| $AUC_{i.v.}$ ($\mu g.ml^{-1}.min$) | | | | | |
| control | 445 | 405 | 126 | 405 | 126 |
| phenobarbitone | 227 | 226 | 89 | 226 | 89 |
| phenobarbitone / control | 0.51 | 0.56 | 0.71 | 0.56 | 0.71 |
| $AUC_{p.o.}$ ($\mu g.ml^{-1}.min$) | | | | | |
| control | 103 | 108 | 33 | 108 | 33 |
| phenobarbitone | 33 | 32 | 12 | 32 | 12 |
| phenobarbitone / control | 0.32 | 0.30 | 0.36 | 0.30 | 0.38 |
| Tocainide | | | | | |
| $AUC_{i.v.}$ ($\mu g.ml^{-1}.min$) | | | | | |
| control | 4002 | 18687 | 5792 | 19013 | 6189 |
| phenobarbitone | 1828 | 4254 | 1675 | 4253 | 1673 |
| phenobarbitone / control | 0.46 | 0.23 | 0.27 | 0.21 | 0.27 |
| $AUC_{p.o.}$ ($\mu g.ml^{-1}.min$) | | | | | |
| control | 4303 | 22727 | 7042 | 24520 | 7610 |
| phenobarbitone | 1827 | 3834 | 1510 | 3819 | 1508 |
| phenobarbitone / control | 0.42 | 0.17 | 0.21 | 0.16 | 0.20 |

# APPENDICES

Appendix 1 Blood concentration-time data of lignocaine following intravenous administration of 2.5, 5.0 and 10.0 mg.kg

| Dose | Rat | Body weight | Blood concentration (μg/ml) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| (mg.kg) | No | (g) | 5 | 10 | 20 | 40 | 60 | 90 | 120 | 180 min |
| 2.5 | 1 | 385 | 2.45 | 1.77 | 1.19 | 0.80 | 0.54 | 0.44 | 0.35 | 0.11 |
| | 2 | 380 | 2.77 | 2.55 | 1.81 | 1.11 | 0.65 | 0.29 | 0.23 | 0.16 |
| | 3 | 385 | 2.58 | 2.27 | 1.96 | 1.46 | 1.14 | 1.02 | 0.80 | 0.31 |
| | 4 | 370 | 3.58 | 2.36 | 1.84 | 1.40 | 1.04 | 0.62 | 0.54 | 0.16 |
| | 5 | 425 | 5.74 | 4.15 | 2.23 | 1.61 | 0.95 | 1.11 | 0.55 | 0.31 |
| | 6 | 425 | 1.87 | 1.60 | 1.29 | 1.00 | 0.75 | 0.54 | 0.27 | 0.11 |
| | Mean±SEM | 395±9.7 | 3.16 | 2.45 | 1.72 | 1.23 | 0.84 | 0.67 | 0.46 | 0.19 |
| | | | ± 0.56 | ± 0.37 | ±0.16 | ±0.13 | ±0.09 | ±0.13 | ±0.09 | ±0.04 |
| 5.0 | 1 | 425 | 8.44 | 8.04 | 7.65 | 5.49 | 4.23 | 2.23 | 1.76 | 0.96 |
| | 2 | 380 | 5.78 | 4.02 | 3.01 | 2.33 | 1.80 | 1.35 | 0.94 | 0.50 |
| | 3 | 405 | 5.32 | 4.67 | 4.10 | 2.00 | 1.75 | 1.05 | 0.46 | 0.33 |
| | 4 | 400 | 7.96 | 6.47 | 4.59 | 2.92 | 2.14 | 1.16 | 0.77 | 0.40 |
| | 5 | 400 | 6.53 | 5.03 | 4.16 | 2.86 | 2.14 | 1.53 | 1.01 | 0.60 |
| | 6 | 400 | 8.84 | 7.07 | 5.92 | 4.18 | 2.68 | 1.71 | 0.82 | 0.48 |
| | 7 | 380 | 7.14 | 6.20 | 4.70 | 2.91 | 1.92 | 1.16 | 0.51 | 0.25 |
| | 8 | 400 | 8.60 | 6.86 | 5.80 | 4.58 | 3.47 | 2.30 | 1.25 | 0.89 |
| | Mean±SEM | 399±5.13 | 7.32 | 6.04 | 4.99 | 3.41 | 2.52 | 1.56 | 0.94 | 0.55 |
| | | | ± 0.47 | ± 0.48 | ±0.50 | ±0.43 | ±0.32 | ±0.17 | ±0.15 | ±0.09 |
| 10.0 | 1 | 480 | 10.32 | 9.08 | 7.82 | 5.84 | 4.55 | 4.08 | 3.20 | 1.24 |
| | 2 | 455 | 13.96 | 11.37 | 8.61 | 4.58 | 3.28 | 2.00 | 1.30 | 0.40 |
| | 3 | 400 | 12.00 | 9.75 | 7.60 | 3.61 | 2.13 | 1.34 | 0.82 | 0.23 |
| | 4 | 400 | 16.40 | 12.13 | 9.18 | 6.32 | 4.93 | 3.31 | 2.30 | 1.10 |
| | 5 | 400 | 11.92 | 8.94 | 7.44 | 5.20 | 4.06 | 2.82 | 1.78 | 0.88 |
| | 6 | 455 | 14.45 | 10.80 | 8.28 | 7.40 | 4.74 | 1.58 | 2.00 | 0.74 |
| | Mean±SEM | 431±14.6 | 13.66 | 10.34 | 8.15 | 5.49 | 3.94 | 2.52 | 1.90 | 0.76 |
| | | | ± 0.89 | ± 0.53 | ±0.27 | ±0.54 | ±0.44 | ±0.44 | ±0.34 | ±0.16 |

Appendix 2: Blood concentration-time data of intravenous lignocaine (5mg/kg) in rats treated either with phenobarbitone, 3, 4-benzpyrene or with sotalol

| Treatment | Rat No | Body weight (g) | Blood concentration (µg/ml) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 5 | 10 | 20 | 40 | 60 | 90 | 120 | 180 min |
| Phenobarbitone | 1 | 375 | 6.50 | 4.60 | 3.06 | 2.10 | 1.78 | 1.09 | 0.52 | 0.12 |
| | 2 | 425 | 4.79 | 3.36 | 2.52 | 1.28 | 1.10 | 0.63 | 0.42 | 0.19 |
| | 3 | 425 | 2.90 | 2.59 | 1.28 | 0.73 | 0.46 | 0.42 | 0.25 | 0.18 |
| | 4 | 405 | 2.34 | 2.09 | 1.70 | 0.94 | 0.83 | 0.58 | 0.56 | 0.30 |
| | 5 | 410 | 10.67 | 7.62 | 5.14 | 4.31 | 3.19 | 1.68 | 1.45 | 0.65 |
| | 6 | 380 | 5.21 | 2.91 | 2.11 | 1.61 | 1.12 | 0.76 | 0.64 | 0.17 |
| | 7 | 415 | 4.10 | 2.62 | 0.90 | 0.80 | 0.65 | 0.25 | 0.08 | 0.02 |
| | 8 | 350 | 3.97 | 2.60 | 1.80 | 1.14 | 0.97 | 0.65 | 0.25 | 0.10 |
| | 9 | 380 | 0.91 | 0.88 | 0.77 | 0.40 | 0.26 | 0.17 | 0.12 | 0.03 |
| | Mean±SEM | 396±8.6 | 5.00±0.94 | 3.25±0.64 | 2.14±0.45 | 1.48±0.39 | 1.15±0.29 | 0.69±0.15 | 0.48±0.14 | 0.19±0.06 |
| 3,4 benzpyrene | 1 | 435 | 5.28 | 4.02 | 3.20 | 2.12 | 2.00 | 1.06 | 0.66 | 0.40 |
| | 2 | 480 | 8.60 | 6.78 | 5.08 | 2.72 | 1.84 | 0.76 | 0.54 | 0.20 |
| | 3 | 435 | 5.50 | 3.70 | 2.72 | 1.28 | 0.86 | 0.38 | 0.30 | 0.20 |
| | 4 | 475 | 8.60 | 6.86 | 6.53 | 4.58 | 3.47 | 3.00 | 1.25 | 0.89 |
| | 5 | 460 | 5.21 | 2.91 | 2.11 | 1.61 | 1.12 | 0.76 | 0.64 | 0.27 |
| | 6 | 475 | 6.64 | 4.79 | 3.99 | 2.46 | 1.86 | 1.25 | 0.68 | 0.37 |
| | Mean±SEM | 460±8.4 | 6.64±0.65 | 4.84±0.67 | 3.94±0.67 | 2.46±0.47 | 1.86±0.37 | 1.20±0.38 | 0.68±0.13 | 0.39±0.11 |
| Sotalol | 1 | 400 | 10.83 | 9.37 | 7.86 | 7.08 | 5.30 | 4.08 | 3.07 | 1.41 |
| | 2 | 375 | 5.58 | 4.45 | 2.87 | 2.07 | 1.36 | 1.08 | 0.81 | 0.46 |
| | 3 | 360 | 10.09 | 7.37 | 5.78 | 5.09 | 3.74 | 2.89 | 2.17 | 1.30 |
| | 4 | 410 | 10.72 | 9.20 | 7.03 | 6.62 | 4.96 | 3.81 | 2.88 | 1.90 |
| | 5 | 380 | 9.29 | 6.91 | 5.36 | 4.58 | 3.33 | 2.58 | 1.94 | 1.10 |
| | 6 | 390 | 7.40 | 6.73 | 5.22 | 3.61 | 3.40 | 2.70 | 2.20 | 1.46 |
| | Mean±SEM | 386±7.3 | 8.98±0.85 | 7.34±0.74 | 5.69±0.70 | 4.84±0.76 | 3.68±0.57 | 2.86±0.43 | 2.18±0.33 | 1.27±0.19 |

Appendix 3: Blood concentration-time data of lignocaine following oral administration of 50, 70 and 90 mg/kg

| Dose (mg/kg) | Rat No | Body weight (g) | Blood concentration (µg/ml) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 10 | 20 | 30 | 45 | 60 | 90 | 120 | 180 min |
| 50 | 1 | 470 | 0.26 | 0.85 | 0.79 | 0.67 | 0.54 | 0.39 | 0.26 | 0.10 |
| | 2 | 475 | 0.96 | 0.86 | 0.79 | 0.78 | 0.65 | 0.35 | 0.15 | 0.10 |
| | 3 | 430 | 0.82 | 0.99 | 0.87 | 0.75 | 0.70 | 0.54 | 0.39 | 0.21 |
| | 4 | 415 | 0.05 | 0.64 | 0.67 | 0.80 | 0.74 | 0.52 | 0.36 | 0.21 |
| | 5 | 390 | 0.46 | 0.33 | 0.29 | 0.23 | 0.18 | 0.10 | 0.06 | 0.02 |
| | 6 | 435 | 0.11 | 0.27 | 0.29 | 0.36 | 0.44 | 0.41 | 0.29 | 0.14 |
| | 7 | 400 | 0.29 | 0.51 | 0.55 | 0.67 | 0.48 | 0.38 | 0.23 | 0.11 |
| | 8 | 400 | 0.32 | 0.35 | 0.32 | 0.28 | 0.23 | 0.22 | 0.15 | 0.11 |
| | Mean ± SEM | 427 ± 11.3 | 0.41 ±0.11 | 0.60 ±0.10 | 0.57 ±0.09 | 0.56 ±0.08 | 0.50 ±0.08 | 0.36 ±0.05 | 0.24 ±0.04 | 0.13 ±0.03 |
| 70 | 1 | 470 | 0.29 | 0.60 | 0.53 | 0.53 | 0.52 | 0.41 | 0.20 | 0.12 |
| | 2 | 430 | 1.00 | 1.23 | 1.18 | 1.06 | 0.80 | 0.54 | 0.45 | 0.18 |
| | 3 | 450 | 1.25 | 1.08 | 1.02 | 0.80 | 0.72 | 0.60 | 0.46 | 0.20 |
| | 4 | 380 | 1.29 | 1.18 | 1.06 | 1.10 | 0.82 | 0.55 | 0.40 | 0.12 |
| | 5 | 355 | 0.36 | 0.63 | 0.59 | 0.52 | 0.46 | 0.44 | 0.19 | 0.06 |
| | 6 | 400 | 0.25 | 0.66 | 0.70 | 0.40 | 0.32 | 0.24 | 0.18 | 0.10 |
| | 7 | 400 | 0.85 | 0.97 | 0.91 | 0.80 | 0.68 | 0.52 | 0.41 | 0.17 |
| | 8 | 425 | 0.27 | 0.68 | 0.85 | 0.70 | 0.60 | 0.47 | 0.28 | 0.12 |
| | Mean ± SEM | 414 ± 13.2 | 0.69 ±0.16 | 0.88 ±0.09 | 0.85 ±0.08 | 0.73 ±0.09 | 0.61 ±0.06 | 0.47 ±0.04 | 0.32 ±0.04 | 0.13 ±0.02 |
| 90 | 1 | 450 | 1.18 | 1.38 | 1.25 | 1.08 | 0.99 | 0.74 | 0.52 | 0.25 |
| | 2 | 425 | 1.45 | 1.26 | 1.18 | 1.10 | 0.85 | 0.64 | 0.54 | 0.30 |
| | 3 | 400 | 1.09 | 1.25 | 1.63 | 1.49 | 1.03 | 0.45 | 0.39 | 0.19 |
| | 4 | 380 | 0.75 | 0.87 | 0.99 | 1.16 | 1.08 | 0.64 | 0.53 | 0.26 |
| | 5 | 410 | 1.11 | 1.56 | 1.55 | 1.40 | 1.39 | 0.66 | 0.58 | 0.23 |
| | 6 | 400 | 0.14 | 0.24 | 0.72 | 1.79 | 1.87 | 1.11 | 0.81 | 0.39 |
| | 7 | 400 | 0.35 | 0.65 | 0.88 | 1.24 | 1.33 | 0.84 | 0.53 | 0.38 |
| | 8 | 375 | 0.34 | 0.67 | 0.85 | 1.00 | 0.91 | 0.74 | 0.64 | 0.32 |
| | Mean ± SEM | 405 ± 8.5 | 0.83 ±0.18 | 0.96 ±0.15 | 1.11 ±0.12 | 1.29 ±0.09 | 1.18 ±0.12 | 0.73 ±0.07 | 0.57 ±0.04 | 0.29 ±0.02 |

Appendix 4: Blood concentration-time data of oral lignocaine (70 mg/kg) in rats treated either with phenobarbitone, 3, 4-benzpyrene or with sotalol

| Treatment | Rat No | Body weight (g) | Blood concentration (µg/ml) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 10 | 20 | 30 | 45 | 60 | 90 | 120 | 180 min |
| Phenobarbitone | 1 | 445 | 0.41 | 0.47 | 0.62 | 0.49 | 0.38 | 0.31 | 0.24 | 0.14 |
| | 2 | 350 | 0.14 | 0.18 | 0.25 | 0.15 | 0.11 | 0.09 | 0.05 | 0.02 |
| | 3 | 425 | 0.16 | 0.20 | 0.17 | 0.17 | 0.13 | 0.11 | 0.10 | 0.03 |
| | 4 | 455 | 0.24 | 0.38 | 0.18 | 0.12 | 0.11 | 0.10 | 0.09 | 0.04 |
| | 5 | 425 | 0.25 | 0.31 | 0.27 | 0.25 | 0.18 | 0.10 | 0.08 | 0.04 |
| | 6 | 450 | 0.21 | 0.27 | 0.32 | 0.29 | 0.18 | 0.14 | 0.10 | 0.06 |
| | Mean ± SEM | 425 ± 15.9 | 0.23 ±0.04 | 0.30 ±0.04 | 0.30 ±0.07 | 0.24 ±0.05 | 0.18 ±0.04 | 0.14 ±0.03 | 0.11 ±0.03 | 0.05 ±0.02 |
| 3, 4 benzpyrene | 1 | 465 | 0.90 | 1.18 | 0.86 | 0.67 | 0.58 | 0.43 | 0.29 | 0.11 |
| | 2 | 475 | 0.65 | 1.15 | 1.00 | 0.75 | 0.54 | 0.31 | 0.21 | 0.10 |
| | 3 | 440 | 0.41 | 0.60 | 0.47 | 0.43 | 0.38 | 0.28 | 0.20 | 0.10 |
| | 4 | 455 | 0.29 | 0.50 | 0.53 | 0.45 | 0.32 | 0.21 | 0.13 | 0.06 |
| | 5 | 450 | 0.49 | 0.79 | 0.66 | 0.53 | 0.42 | 0.31 | 0.21 | 0.09 |
| | 6 | 430 | 0.22 | 0.51 | 0.43 | 0.33 | 0.28 | 0.20 | 0.14 | 0.08 |
| | Mean ± SEM | 452 ± 6.7 | 0.49 ±0.10 | 0.79 ±0.13 | 0.66 ±0.09 | 0.53 ±0.06 | 0.42 ±0.05 | 0.29 ±0.03 | 0.20 ±0.02 | 0.09 ±0.01 |
| Sotalol | 1 | 425 | 1.24 | 1.29 | 1.10 | 1.03 | 0.92 | 0.88 | 0.73 | 0.53 |
| | 2 | 355 | 1.34 | 1.30 | 0.97 | 0.73 | 0.70 | 0.58 | 0.52 | 0.31 |
| | 3 | 395 | 1.25 | 2.01 | 1.83 | 1.45 | 1.35 | 1.27 | 1.06 | 0.65 |
| | 4 | 400 | 0.85 | 0.97 | 0.91 | 0.80 | 0.70 | 0.62 | 0.49 | 0.35 |
| | 5 | 410 | 0.62 | 0.64 | 0.55 | 0.52 | 0.46 | 0.44 | 0.40 | 0.33 |
| | 6 | 395 | 0.74 | 0.80 | 0.73 | 0.66 | 0.58 | 0.53 | 0.43 | 0.31 |
| | Mean ± SEM | 397 ± 9.5 | 1.01 ±0.12 | 1.17 ±0.20 | 1.01 ±0.18 | 0.86 ±0.13 | 0.78 ±0.13 | 0.72 ±0.12 | 0.60 ±0.10 | 0.41 ±0.06 |

Appendix 5: Blood concentration-time data of tocainide following intravenous administration of 5, 20, 35 and 50 mg/kg

| Dose (mg/kg) | Rat No | Body weight (g) | Blood concentration (µg/ml) 5 | 15 | 30 | 60 | 120 | 180 | 240 | 360 | 540 | 720 min |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 5 | 1 | 465 | 9.81 | 5.63 | 4.31 | 3.44 | 1.69 | 0.69 | 0.50 | 0.11 | — | — |
| | 2 | 500 | 12.50 | 8.80 | 3.48 | 1.93 | 1.06 | 0.75 | 0.44 | 0.23 | — | — |
| | 3 | 490 | 7.60 | 4.36 | 2.28 | 1.50 | 1.10 | 0.73 | 0.55 | 0.27 | — | — |
| | 4 | 470 | 11.16 | 7.21 | 3.89 | 2.68 | 1.38 | 0.72 | 0.47 | 0.17 | — | — |
| | 5 | 480 | 10.00 | 6.58 | 2.88 | 1.72 | 1.08 | 0.74 | 0.50 | 0.25 | — | — |
| | Mean ± SEM | 481 ± 6.4 | 10.21±0.81 | 6.52±0.75 | 3.37 ±0.36 | 2.25 ±0.36 | 1.26 ±0.12 | 0.73 ±0.01 | 0.49 ±0.02 | 0.21 ±0.03 | — | — |
| 20 | 1 | 490 | 30.44 | 17.44 | 9.13 | 6.00 | 4.38 | — | 2.19 | 1.10 | 0.42 | 0.16 |
| | 2 | 500 | 29.61 | 18.75 | 12.20 | 9.06 | 5.53 | — | 2.78 | 1.78 | 0.72 | 0.33 |
| | 3 | 470 | 21.60 | 18.17 | 12.04 | 9.00 | 6.23 | — | 3.96 | 1.39 | 0.63 | 0.36 |
| | 4 | 490 | 30.02 | 18.10 | 10.66 | 7.53 | 4.95 | — | 2.48 | 1.44 | 0.57 | 0.25 |
| | 5 | 485 | 25.60 | 18.46 | 12.12 | 8.98 | 5.88 | — | 3.37 | 1.58 | 0.67 | 0.34 |
| | Mean ± SEM | 487 ± 4.9 | 27.45 ±1.70 | 18.18 ±0.22 | 11.23 ±0.60 | 8.11 ±0.60 | 5.39 ±0.33 | — | 2.96 ±0.32 | 1.46 ±0.11 | 0.60 ±0.05 | 0.29 ±0.04 |
| 35 | 1 | 575 | 26.60 | 24.58 | 19.40 | 17.44 | 13.95 | — | 6.00 | 3.39 | 1.83 | 0.55 |
| | 2 | 530 | 36.34 | 19.32 | 16.80 | 13.50 | 8.55 | — | 4.55 | 2.50 | 1.83 | 0.50 |
| | 3 | 500 | 31.47 | 21.95 | 18.10 | 13.00 | 11.25 | — | 5.33 | 2.94 | 1.81 | 0.53 |
| | 4 | 495 | 27.60 | 25.48 | 18.42 | 16.24 | 12.81 | — | 4.56 | 2.48 | 1.86 | 0.50 |
| | 5 | 490 | 31.07 | 21.51 | 17.30 | 14.00 | 10.42 | — | 4.95 | 2.82 | 1.60 | 0.60 |
| | Mean ± SEM | 518 ± 15.9 | 30.61 ±1.72 | 22.57 ±1.11 | 18.00 ±0.45 | 14.84 ±0.85 | 11.40 ±0.94 | — | 5.08 ±0.27 | 2.83 ±0.17 | 1.79 ±0.05 | 0.54 ±0.02 |
| 50 | 1 | 540 | 53.99 | 45.43 | 30.11 | 22.49 | 15.58 | — | 9.91 | 3.48 | 1.57 | 0.90 |
| | 2 | 550 | 48.04 | 36.79 | 25.13 | 22.26 | 13.29 | — | 7.62 | 3.29 | 1.63 | 1.14 |
| | 3 | 500 | 51.01 | 41.11 | 27.62 | 22.37 | 14.43 | — | 8.76 | 3.38 | 1.60 | 1.02 |
| | 4 | 495 | 44.95 | 31.35 | 25.85 | 18.57 | 16.07 | — | 7.61 | 4.19 | 2.58 | 0.76 |
| | 5 | 470 | 49.47 | 38.39 | 27.98 | 20.53 | 15.82 | — | 8.76 | 3.83 | 2.07 | 0.83 |
| | Mean ± SEM | 511 ± 14.9 | 49.49 ±1.50 | 38.61 ±2.33 | 27.34 ±0.87 | 21.44 ±0.85 | 15.04 ±0.52 | — | 8.53 ±0.43 | 3.63 ±0.17 | 1.89 ±0.19 | 0.93 ±0.07 |

Appendix 6: Blood concentration-time data of intravenous tocainide (35 mg/kg) in rats treated either with phenobarbitone, 3,4 benzpyrene or with sotalol

| Treatment | Rat no | Body weight (g) | Blood concentration (µg/ml) 5 | 15 | 30 | 60 | 120 | 240 | 360 | 540 | 720 min |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Phenobarbitone | 1 | 525 | 37.05 | 23.62 | 15.45 | 7.86 | 3.87 | 1.18 | 0.47 | 0.08 | — |
| | 2 | 500 | 37.87 | 23.08 | 16.45 | 10.71 | 7.83 | 3.54 | 1.70 | 0.70 | — |
| | 3 | 490 | 33.61 | 24.31 | 16.50 | 10.33 | 5.31 | 1.62 | 0.96 | 0.43 | — |
| | 4 | 500 | 34.84 | 23.67 | 16.13 | 9.63 | 5.67 | 2.11 | 1.04 | 0.41 | — |
| | 5 | 470 | 26.60 | 21.95 | 14.31 | 6.86 | 3.00 | 1.62 | 0.92 | 0.28 | — |
| | 6 | 485 | 36.59 | 23.32 | 15.77 | 8.00 | 3.12 | 1.10 | 0.46 | 0.08 | — |
| | Mean ± SEM | 495 ± 7.5 | 34.43 | 23.32 | 15.77 | 8.89 | 4.80 | 1.86 | 0.92 | 0.30 | — |
| | | | ±1.69 | ±0.32 | ±0.33 | ±0.63 | ±0.75 | ±0.37 | ±0.19 | ±0.11 | |
| 3,4-benzpyrene | 1 | 400 | 33.84 | 23.67 | 16.13 | 9.63 | 5.67 | 2.11 | 1.04 | 0.36 | 0.13 |
| | 2 | 420 | 42.41 | 29.80 | 19.12 | 8.78 | 4.12 | 2.22 | 1.34 | 0.98 | 0.28 |
| | 3 | 440 | 22.94 | 16.90 | 12.04 | 7.01 | 4.57 | 2.56 | 1.00 | 0.80 | 0.20 |
| | 4 | 410 | 34.83 | 19.25 | 13.65 | 12.48 | 5.60 | 3.95 | 1.91 | 0.82 | 0.25 |
| | 5 | 400 | 28.86 | 18.08 | 12.85 | 9.75 | 5.09 | 3.26 | 1.50 | 0.50 | 0.13 |
| | 6 | 460 | 35.85 | 20.87 | 14.49 | 9.69 | 5.38 | 2.68 | 1.27 | 0.43 | 0.10 |
| | Mean ± SEM | 421 ± 9.8 | 33.12 | 21.43 | 14.71 | 9.56 | 5.07 | 2.80 | 1.34 | 0.65 | 0.18 |
| | | | ±2.70 | ±1.93 | ±1.05 | ±0.72 | ±0.25 | ±0.28 | ±0.14 | ±0.10 | ±0.03 |
| Sotalol | 1 | 350 | 29.50 | 25.33 | 22.50 | 21.58 | 18.55 | 9.00 | 6.80 | 3.56 | 2.50 |
| | 2 | 350 | 26.70 | 19.93 | 18.11 | 14.58 | 11.25 | 7.97 | 5.80 | 3.53 | 2.68 |
| | 3 | 360 | 30.00 | 23.57 | 21.50 | 18.06 | 12.35 | 5.24 | 3.43 | 2.34 | 1.00 |
| | 4 | 490 | 31.18 | 21.00 | 17.30 | 13.79 | 10.42 | 4.97 | 2.83 | 1.60 | 0.62 |
| | 5 | 385 | 28.80 | 24.52 | 19.96 | 17.15 | 12.58 | 4.90 | 2.95 | 2.10 | 0.75 |
| | 6 | 370 | 30.34 | 23.16 | 19.90 | 17.68 | 14.48 | 6.98 | 4.81 | 2.58 | 1.56 |
| | Mean ± SEM | 384 ± 21.8 | 29.42 | 22.92 | 21.40 | 17.14 | 13.27 | 6.51 | 4.44 | 2.62 | 1.52 |
| | | | ±0.63 | ±0.85 | ±1.02 | ±1.13 | ±1.19 | ±0.71 | ±0.67 | ±0.32 | ±0.36 |

Appendix 7: Blood concentration-time data of tocainide following oral administration of 50, 100, 200, 300 and 400 mg.kg

| Dose (mg/kg) | Rat No | Body weight (g) | Blood concentration (µg/ml) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 0.5 | 1 | 2.5 | 4 | 6 | 9 | 12 | 18 | 24 | 30 | 36 | 48 hrs |
| 50 | 1 | 510 | 0.93 | 1.72 | 9.13 | 3.75 | 2.45 | 0.90 | 0.40 | 0.18 | — | — | — | — |
| | 2 | 560 | 3.96 | 4.51 | 7.87 | 7.15 | 4.91 | 3.51 | 2.63 | 0.98 | 0.39 | 0.20 | — | — |
| | 3 | 575 | 2.40 | 2.91 | 5.45 | 5.70 | 5.55 | 4.68 | 3.71 | 1.11 | 0.43 | 0.25 | — | — |
| | 4 | 510 | 4.64 | 6.07 | 7.17 | 6.00 | 5.45 | 2.71 | 2.43 | 1.44 | 0.48 | 0.22 | — | — |
| | 5 | 500 | 3.18 | 3.71 | 6.72 | 6.42 | 5.23 | 4.10 | 3.17 | 1.04 | 0.41 | 0.23 | — | — |
| | 6 | 490 | 4.25 | 4.60 | 6.59 | 7.80 | 7.57 | 5.32 | 4.33 | 2.00 | 0.50 | 0.26 | — | — |
| | Mean±SEM | 524±14.2 | 3.23 ±0.56 | 3.90 ±0.61 | 7.15 ±0.51 | 6.14 ±0.57 | 5.19 ±0.67 | 3.54 ±0.64 | 2.78 ±0.55 | 1.12 ±0.24 | 0.37 ±0.07 | 0.21 ±0.05 | — | — |
| 100 | 1 | 500 | 5.24 | 6.56 | 9.13 | 11.50 | 10.84 | 9.65 | 7.48 | 4.55 | 2.75 | 2.02 | 1.01 | — |
| | 2 | 510 | 9.28 | 12.14 | 14.35 | 12.20 | 10.89 | 7.50 | 4.86 | 2.87 | 1.50 | 1.20 | 0.80 | — |
| | 3 | 480 | 6.36 | 7.42 | 13.44 | 12.84 | 10.46 | 8.20 | 6.34 | 2.09 | 1.70 | 1.10 | 0.85 | — |
| | 4 | 490 | 7.26 | 9.35 | 11.74 | 11.85 | 10.87 | 7.54 | 6.17 | 3.71 | 2.12 | 1.61 | 0.90 | — |
| | Mean±SEM | 495±6.4 | 7.03 ±0.85 | 8.87 ±1.24 | 12.16 ±1.15 | 12.10 ±0.28 | 10.76 ±0.10 | 8.22 ±0.50 | 6.21 ±0.54 | 3.30 ±0.53 | 2.02 ±0.28 | 1.48 ±0.21 | 0.89 ±0.04 | — |
| 200 | 1 | 460 | 9.40 | 9.74 | 16.45 | 14.24 | 13.03 | 10.83 | 9.77 | 5.28 | 3.87 | 1.94 | 1.30 | — |
| | 2 | 510 | 8.50 | 9.18 | 13.18 | 15.82 | 17.53 | 10.64 | 8.66 | 7.52 | 7.94 | 3.73 | 2.85 | — |
| | 3 | 490 | 7.86 | 9.84 | 13.70 | 17.25 | 16.26 | 14.48 | 11.22 | 6.80 | 4.12 | 3.03 | 2.20 | — |
| | 4 | 500 | 8.95 | 9.46 | 14.81 | 15.03 | 15.28 | 10.73 | 9.22 | 6.40 | 4.43 | 2.83 | 2.01 | — |
| | Mean±SEM | 490±10.8 | 8.68 ±0.33 | 9.55 ±0.15 | 14.53 ±0.72 | 15.85 ±0.64 | 15.52 ±0.95 | 11.67 ±0.94 | 9.72 ±0.55 | 6.50 ±0.47 | 5.09 ±0.96 | 2.88 ±0.37 | 2.09 ±0.32 | — |

contd.....

Appendix 7: (contd.)

| Dose (mg/kg) | Rat no | Body weight (g) | Blood concentration (µg/ml) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 0.5 | 1 | 2.5 | 4 | 6 | 9 | 12 | 18 | 24 | 30 | 36 | 38 hrs |
| 300 | 1 | 530 | 19.00 | 35.42 | 30.69 | 24.51 | 27.01 | 22.56 | 15.32 | 7.87 | 5.17 | 4.44 | 4.85 | 2.57 |
| | 2 | 495 | 15.72 | 19.58 | 27.39 | 34.50 | 32.52 | 28.95 | 22.44 | 13.65 | 8.25 | 6.06 | 3.03 | 1.25 |
| | 3 | 490 | 17.36 | 27.50 | 29.04 | 29.50 | 29.77 | 25.76 | 18.88 | 10.76 | 6.71 | 5.25 | 3.94 | 1.91 |
| | 4 | 500 | 13.43 | 14.20 | 22.21 | 22.54 | 22.92 | 16.10 | 13.83 | 9.60 | 6.64 | 4.25 | 3.01 | 1.60 |
| | Mean±SEM | 504±9.0 | 16.38 | 24.20 | 27.33 | 27.76 | 28.05 | 23.34 | 17.62 | 10.47 | 6.69 | 5.00 | 3.71 | 1.83 |
| | | | ±1.19 | ±4.63 | ±1.83 | ±2.68 | ±2.05 | ±2.74 | ±1.92 | ±1.21 | ±0.63 | ±0.41 | ±0.44 | ±0.28 |
| 400 | 1 | 525 | 33.69 | 47.79 | 41.16 | 66.54 | 51.78 | 34.02 | 32.60 | 17.72 | 11.10 | 5.85 | 3.73 | 1.48 |
| | 2 | 550 | 34.00 | 42.16 | 47.79 | 67.00 | 52.00 | 35.02 | 33.00 | 17.70 | 12.32 | 6.12 | 4.00 | 1.80 |
| | 3 | 510 | 37.12 | 48.60 | 57.40 | 48.80 | 43.57 | 34.72 | 25.18 | 13.42 | 10.88 | 6.41 | 4.59 | 1.62 |
| | 4 | 500 | 25.33 | 47.23 | 40.92 | 36.01 | 32.68 | 30.08 | 20.43 | 10.49 | 6.89 | 5.92 | 6.47 | 3.43 |
| | Mean±SEM | 521±10.9 | 32.53 | 46.44 | 46.82 | 54.59 | 45.51 | 33.46 | 27.80 | 14.84 | 10.30 | 6.07 | 4.70 | 2.08 |
| | | | ±2.52 | ±1.45 | ±3.87 | ±7.50 | ±4.10 | ±1.14 | ±3.04 | ±1.77 | ±1.18 | ±0.12 | ±0.62 | ±0.45 |

Appendix 8: Blood concentration-time data of tocainide following intraperitoneal administration of 50 mg/kg

| Dose (mg/kg) | Rat no | Body weight (g) | Blood concentration (µg/ml) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | 0.5 | 1 | 2.5 | 4 | 6 | 9 | 12 hrs |
| 50 | 1 | 475 | 8.98 | 10.03 | 11.11 | 7.21 | 4.83 | 2.78 | 1.46 |
| | 2 | 455 | 8.87 | 10.17 | 9.32 | 7.53 | 3.99 | 2.27 | 1.06 |
| | 3 | 495 | 10.10 | 13.20 | 10.77 | 9.02 | 5.75 | 3.13 | 2.13 |
| | 4 | 495 | 9.31 | 11.13 | 10.40 | 7.92 | 4.86 | 2.73 | 1.55 |
| | 5 | 480 | 9.48 | 11.68 | 10.04 | 8.27 | 4.87 | 2.70 | 1.59 |
| | 6 | 500 | 8.69 | 10.10 | 10.86 | 7.80 | 4.00 | 2.30 | 1.28 |
| | Mean ± SEM | 483 ± 24.2 | 9.24 | 11.05 | 10.42 | 7.96 | 4.72 | 2.65 | 1.51 |
| | | | ±0.21 | ±0.51 | ±0.27 | ±0.26 | ±0.27 | ±0.13 | ±0.15 |

Appendix 9: Blood concentration-time data of oral tocainide (50 mg/kg) in rats treated either with phenobarbitone, 3,4-benzpyrene or with sotalol

| Treatment | Rat no | Body weight (g) | Blood concentration (µg/ml) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | 0.5 | 1 | 2.5 | 4 | 6 | 9 | 12 hrs |
| Phenobarbitone | 1 | 470 | 3.00 | 3.58 | 7.27 | 3.26 | 2.74 | 1.79 | 0.70 |
| | 2 | 460 | 2.95 | 3.14 | 4.65 | 3.19 | 2.75 | 1.45 | 0.80 |
| | 3 | 490 | 1.27 | 1.41 | 2.21 | 2.16 | 1.71 | 1.06 | 0.45 |
| | 4 | 485 | 2.41 | 2.71 | 4.71 | 2.87 | 2.41 | 1.59 | 0.57 |
| | 5 | 475 | 2.25 | 2.50 | 4.25 | 3.10 | 2.80 | 1.30 | 0.70 |
| | 6 | 470 | 1.76 | 1.95 | 3.23 | 2.63 | 2.25 | 1.18 | 0.72 |
| | Mean±SEM | 475±4.5 | 2.44 | 2.55 | 4.39 | 2.87 | 2.44 | 1.39 | 0.66 |
| | | | ±0.26 | ±0.32 | ±0.70 | ±0.17 | ±0.17 | ±0.11 | ±0.05 |
| 3,4-benzpyrene | 1 | 400 | 2.29 | 3.36 | 5.50 | 4.95 | 3.27 | 1.62 | 1.20 |
| | 2 | 410 | 2.74 | 3.38 | 4.17 | 3.87 | 3.02 | 1.72 | 1.00 |
| | 3 | 400 | 2.45 | 3.14 | 4.24 | 2.48 | 1.90 | 1.06 | 0.59 |
| | 4 | 450 | 2.30 | 3.69 | 5.50 | 4.43 | 3.51 | 1.98 | 1.15 |
| | 5 | 430 | 2.45 | 3.39 | 4.85 | 3.94 | 2.92 | 1.60 | 0.66 |
| | 6 | 480 | 2.95 | 3.14 | 4.65 | 3.19 | 2.75 | 1.45 | 0.80 |
| | Mean±SEM | 428±13.0 | 2.53 | 3.35 | 4.82 | 3.81 | 2.89 | 1.57 | 0.90 |
| | | | ±0.11 | ±0.08 | ±0.24 | ±0.36 | ±0.23 | ±0.12 | ±0.10 |
| Sotalol | 1 | 400 | 5.04 | 6.53 | 7.96 | 4.81 | 3.01 | 2.52 | 1.73 |
| | 2 | 380 | 5.26 | 7.06 | 6.76 | 6.45 | 6.00 | 4.21 | 3.16 |
| | 3 | 400 | 2.14 | 2.56 | 5.61 | 3.75 | 2.40 | 1.20 | 0.64 |
| | 4 | 395 | 4.61 | 5.78 | 7.32 | 6.80 | 5.45 | 3.86 | 2.89 |
| | 5 | 430 | 3.96 | 4.50 | 7.88 | 7.15 | 4.91 | 3.51 | 2.63 |
| | 6 | 410 | 4.15 | 5.38 | 6.78 | 5.38 | 4.42 | 3.10 | 2.20 |
| | Mean±SEM | 402±6.8 | 4.19 | 5.30 | 7.05 | 5.72 | 4.36 | 3.07 | 2.21 |
| | | | ±0.46 | ±0.66 | ±0.36 | ±0.53 | ±0.57 | ±0.44 | ±0.37 |

Appendix 10. Blood concentration-time data following the first and the seventh doses of tocainide 20 mg.kg$^{-1}$ intravenously every 8 hours

| | Rat no | Body weight (g) | Blood concentration (µg/ml) | | | | |
|---|---|---|---|---|---|---|---|
| | | | 0 | 120 | 240 | 360 | 480 min |
| After the first dose | 1 | 570 | —— | 5.45 | 2.57 | 1.47 | 1.38 |
| | 2 | 540 | —— | 7.10 | 4.62 | 1.87 | 1.63 |
| | 3 | 500 | —— | 7.93 | 5.11 | 1.65 | 1.00 |
| | 4 | 450 | —— | 7.51 | 4.87 | 1.76 | 1.31 |
| | 5 | 460 | —— | 6.69 | 3.84 | 1.56 | 1.19 |
| | 6 | 450 | —— | 7.00 | 4.81 | 1.90 | 1.64 |
| | Mean±SEM | 495±20.8 | —— | 6.94±0.35 | 4.30±0.39 | 1.70±0.07 | 1.36±0.10 |
| After the seventh dose | 1 | | 1.50 | 8.45 | 5.12 | 2.01 | 1.78 |
| | 2 | | 2.80 | 10.09 | 5.64 | 4.30 | 3.08 |
| | 3 | | 3.42 | 11.49 | 8.37 | 6.83 | 5.79 |
| | 4 | | 1.52 | 8.43 | 5.20 | 1.98 | 1.80 |
| | 5 | | 2.46 | 9.97 | 5.60 | 3.12 | 1.99 |
| | 6 | | 2.91 | 9.98 | 5.62 | 3.80 | 2.60 |
| | Mean±SEM | | 2.43±0.32 | 9.73±0.47 | 5.92±0.50 | 3.67±0.74 | 2.84±0.62 |

Appendix 11 Blood concentration-time data following the first and the tenth dose of tocainide 200 $mg.kg^{-1}$ by mouth every 12 hours

| | Rat no | Body weight (g) | Blood concentration (µg/ml) 0 | 0.5 | 1 | 2.5 | 4 | 6 | 9 | 12 hrs |
|---|---|---|---|---|---|---|---|---|---|---|
| After the first dose | 1 | 475 | — | 8.98 | 8.12 | 11.10 | 23.21 | 15.79 | 13.20 | 10.90 |
| | 2 | 495 | — | 4.85 | 5.29 | 8.82 | 10.59 | 11.69 | 12.92 | 10.13 |
| | 3 | 480 | — | 7.47 | 7.72 | 8.55 | 11.63 | 10.30 | 9.82 | 7.92 |
| | 4 | 475 | — | 7.72 | 11.38 | 15.15 | 18.38 | 13.00 | 11.32 | 8.01 |
| | 5 | 470 | — | 9.00 | 8.20 | 11.11 | 23.20 | 15.80 | 13.19 | 10.91 |
| | Mean±SEM | 479±4.3 | — | 7.60 ±0.69 | 8.14 ±0.88 | 10.95 ±1.08 | 17.40 ±2.48 | 13.32 ±1.00 | 12.09 ±0.61 | 9.57 ±0.61 |
| After the tenth dose | 1 | | 22.07 | 48.59 | 45.21 | 39.69 | 29.13 | 22.25 | 13.64 | 20.98 |
| | 2 | | 22.69 | 29.49 | 26.83 | 27.34 | 23.05 | 16.26 | 12.03 | 13.82 |
| | 3 | | 18.30 | 28.82 | 32.46 | 32.11 | 30.04 | 22.75 | 23.98 | 26.29 |
| | 4 | | 14.62 | 20.82 | 22.37 | 20.31 | 17.10 | 19.32 | 15.82 | 14.07 |
| | 5 | | 23.00 | 30.56 | 28.83 | 27.63 | 23.15 | 17.82 | 13.04 | 14.12 |
| | Mean±SEM | | 20.14 ±1.62 | 31.66 ±4.57 | 31.14 ±3.88 | 29.42 ±3.11 | 24.49 ±2.35 | 19.69 ±1.25 | 15.55 ±2.16 | 18.21 ±2.39 |

Appendix 12 Cardiac output and regional blood flow in control rats.

| Rat no | Body weight (g) | Mean arterial pressure (mm.Hg) | Cardiac output $ml.min^{-1}$ | Liver | | | | | GI Tract and Pancreas | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Wt (g) | blood flow | | | % CO | Wt (g) | blood flow | | % CO |
| | | | | | $ml.min^{-1}$ | $ml.min^{-1}.g^{-1}$ | $g.100g,bw^{-1}$ | | | $ml.min^{-1}$ | $ml.min^{-1}.g^{-1}$ | |
| 1 | 500 | 110. | 64.04 | 16.62 | 5.78 | 0.35 | 2.77 | 9.03 | 12.74 | 10.18 | 0.80 | 15.90 |
| 2 | 570 | 117.5 | 57.76 | 16.89 | 5.61 | 0.32 | 2.96 | 9.71 | 12.01 | 7.44 | 0.62 | 12.88 |
| 3 | 500 | 132.5 | 69.29 | 17.52 | 2.60 | 0.15 | 3.50 | 3.75 | 18.64 | 13.16 | 0.71 | 18.99 |
| 4 | 595 | 115 | 139.06 | 21.38 | 14.10 | 0.66 | 3.59 | 10.14 | 11.89 | 22.39 | 1.88 | 16.10 |
| 5 | 490 | 100 | 79.16 | 18.88 | 3.38 | 0.19 | 3.85 | 4.27 | 11.98 | 13.72 | 1.15 | 17.33 |
| 6 | 515 | 115 | 123.21 | 19.63 | 3.49 | 0.22 | 3.81 | 2.83 | 10.67 | 17.47 | 1.64 | 14.18 |
| 7 | 525 | 125 | 96.59 | 17.92 | 3.68 | 0.21 | 3.41 | 3.81 | 9.68 | 6.78 | 0.70 | 7.02 |
| 8 | 525 | 105 | 66.97 | 17.02 | 2.88 | 0.17 | 3.24 | 4.30 | 20.05 | 13.44 | 0.67 | 20.07 |
| 9 | 300 | 120 | 60.54 | 12.30 | 2.42 | 0.20 | 4.10 | 3.99 | 15.96 | 12.46 | 0.78 | 20.59 |
| 10 | 595 | 125 | 114.69 | 21.37 | 11.57 | 0.54 | 3.59 | 10.09 | 11.89 | 18.51 | 1.56 | 16.14 |
| Mean | 511.5 | 116.5 | 87.13 | 17.95 | 5.55 | 0.30 | 3.48 | 6.19 | 13.55 | 13.55 | 1.05 | 15.92 |
| ±SEM | 26.5 | 3.10 | 9.27 | 0.84 | 1.28 | 0.05 | 0.13 | 0.98 | 1.10 | 1.54 | 0.15 | 1.26 |

Appendix 12 (contd.)

| Rat | Spleen | | | | Hepatosplanchnic | | | |
|---|---|---|---|---|---|---|---|---|
| | Wt (g) | blood flow | | % CO | blood flow | | | % CO |
| | | $ml.min^{-1}$ | $ml.min^{-1}.g^{-1}$ | | $ml.min^{-1}$ | $ml.min^{-1}.g^{-1}$ | $ml.min^{-1}$ $100g,bw^{-1}$ | |
| 1 | 0.92 | 0.88 | 0.96 | 1.37 | 16.84 | 1.01 | 3.37 | 26.30 |
| 2 | 0.76 | 0.78 | 1.03 | 1.35 | 13.83 | 0.82 | 2.43 | 23.94 |
| 3 | 0.80 | 1.42 | 1.78 | 2.05 | 17.18 | 0.98 | 3.44 | 24.79 |
| 4 | 0.94 | 2.56 | 2.74 | 1.85 | 38.95 | 1.82 | 6.55 | 28.01 |
| 5 | 1.12 | 1.77 | 1.58 | 2.24 | 18.87 | 1.00 | 3.85 | 23.84 |
| 6 | 0.73 | 2.58 | 3.51 | 2.09 | 23.54 | 1.50 | 4.48 | 19.10 |
| 7 | 0.79 | 1.45 | 1.84 | 1.50 | 11.91 | 0.67 | 2.27 | 12.33 |
| 8 | 1.01 | 1.76 | 1.75 | 2.63 | 18.08 | 1.06 | 3.44 | 27.00 |
| 9 | 0.98 | 1.18 | 1.20 | 1.95 | 16.06 | 1.30 | 5.35 | 26.53 |
| 10 | 0.94 | 2.12 | 2.27 | 1.85 | 32.19 | 1.50 | 5.41 | 28.07 |
| Mean | 0.90 | 1.65 | 1.87 | 1.89 | 20.74 | 1.17 | 4.06 | 23.99 |
| ±SEM | 0.04 | 0.20 | 0.25 | 0.13 | 2.70 | 0.11 | 0.43 | 1.54 |

Appendix 12 (contd.)

| Rat | Kidneys | | | | Heart | | | | Lungs | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Wt (g) | blood flow | | % CO | Wt (g) | blood flow | | % CO | Wt (g) | blood flow | | % CO |
| | | $ml.min^{-1}$ | $ml.min^{-1}.g^{-1}$ | | | $ml.min^{-1}$ | $ml.min^{-1}.g^{-1}$ | | | $ml.min^{-1}$ | $ml.min^{-1}g^{-1}$ | |
| 1 | 3.68 | 8.30 | 2.25 | 12.96 | 1.76 | 4.56 | 2.59 | 7.12 | 1.64 | 0.22 | 0.13 | 0.34 |
| 2 | 3.93 | 14.73 | 3.75 | 25.50 | 1.70 | 1.97 | 1.16 | 3.41 | — | — | — | — |
| 3 | 4.08 | 13.31 | 3.26 | 19.21 | 2.42 | 3.01 | 1.24 | 4.34 | 1.57 | 0.56 | 0.36 | 0.81 |
| 4 | 4.41 | 39.31 | 8.87 | 28.13 | 1.98 | 5.98 | 3.02 | 4.30 | 2.00 | 2.06 | 1.03 | 1.48 |
| 5 | 3.21 | 10.20 | 3.18 | 12.89 | 1.49 | 3.10 | 2.07 | 3.92 | 2.13 | 2.39 | 1.12 | 3.02 |
| 6 | 3.57 | 16.53 | 4.63 | 13.42 | 1.44 | 5.76 | 0.70 | 4.67 | 2.09 | 1.66 | 0.79 | 1.35 |
| 7 | 3.56 | 10.18 | 2.86 | 10.54 | 1.53 | 9.01 | 5.89 | 9.33 | 2.63 | 2.11 | 0.80 | 2.18 |
| 8 | 3.74 | 14.22 | 3.80 | 21.23 | 1.46 | 2.45 | 1.67 | 3.66 | 1.73 | 0.79 | 0.46 | 1.18 |
| 9 | 2.86 | 10.18 | 3.56 | 16.83 | 1.05 | 1.67 | 1.59 | 2.76 | 1.53 | 0.71 | 0.46 | 1.17 |
| 10 | 4.41 | 32.34 | 7.33 | 28.20 | 1.98 | 4.95 | 2.50 | 4.31 | 2.00 | 1.70 | 0.85 | 1.48 |
| Mean | 3.74 | 16.91 | 4.35 | 18.89 | 1.68 | 4.25 | 2.24 | 4.78 | 1.92 | 1.35 | 0.67 | 1.44 |
| ±SEM | 0:1 | 3.28 | 0.66 | 2.10 | 0.12 | 0.72 | 0.46 | 0.62 | 0.12 | 0.25 | 0.11 | 0.26 |

Appendix 13 Cardiac output and regional blood flow in phenobarbitone treated rats.

| Rat no | Body weight (g) | Mean arterial pressure (mm.Hg) | Cardiac output $ml.min^{-1}$ | Liver | | | | | GI Tract and Pancreas | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Wt (g) | blood flow | | | % CO | Wt (g) | blood flow | | % CO |
| | | | | | $ml.min^{-1}$ | $ml.min^{-1}.g^{-1}$ | $g.100g,bw^{-1}$ | | | $ml.min^{-1}$ | $ml.min^{-1}.g^{-1}$ | |
| 1 | 525 | 132.5 | 87.68 | 21.99 | 3.46 | 0.16 | 4.07 | 3.95 | 10.97 | 16.71 | 1.52 | 19.06 |
| 2 | 515 | 125 | 145.18 | 26.08 | 4.96 | 0.19 | 5.06 | 3.42 | 19.16 | 14.61 | 0.76 | 10.06 |
| 3 | 500 | 135 | 134.10 | 19.66 | 11.06 | 0.56 | 3.93 | 8.35 | 11.06 | 20.88 | 1.89 | 15.57 |
| 4 | 350 | 135 | 102.69 | 20.68 | 3.26 | 0.16 | 5.91 | 3.17 | 10.79 | 13.53 | 1.25 | 13.17 |
| 5 | 450 | 132.5 | 56.79 | 15.12 | 5.92 | 0.39 | 3.36 | 10.42 | 16.34 | 17.63 | 1.08 | 31.04 |
| 6 | 450 | 112.5 | 66.33 | 18.84 | 3.78 | 0.20 | 4.19 | 5.69 | 14.17 | 19.65 | 1.39 | 29.55 |
| 7 | 540 | 130 | 159.83 | 22.78 | 23.93 | 1.05 | 4.22 | 15.03 | 11.18 | 18.90 | 1.69 | 11.87 |
| 8 | 525 | | | 20.95 | | | 3.99 | | | | | |
| 9 | 525 | | | 25.79 | | | 4.91 | | | | | |
| 10 | 455 | | | 21.55 | | | 4.74 | | | | | |
| Mean | 475.71 | 128.93 | 107.54 | 21.34 | 5.41 | 0.39 | 4.45 | 7.13 | 13.38 | 17.41 | 1.37 | 18.62 |
| ±SEM | 24.79 | 3.03 | 15.06 | 1.02 | 1.20 | 0.12 | 0.23 | 1.66 | 1.25 | 1.01 | 0.14 | 3.21 |

Appendix 13 (contd.

| Rat | Spleen | | | | Hepatosplanchnic | | | |
|---|---|---|---|---|---|---|---|---|
| | Wt (g) | blood flow | | % CO | blood flow | | | % CO |
| | | $ml.min^{-1}$ | $ml.min^{-1}.g^{-1}$ | | $ml.min^{-1}$ | $ml.min^{-1}.g^{-1}$ | $ml.min^{-1}$ $100g,bw^{-1}$ | |
| 1 | 0.98 | 1.34 | 1.36 | 1.53 | 21.51 | 0.98 | 4.09 | 24.65 |
| 2 | 0.70 | 1.18 | 1.68 | 0.81 | 20.75 | 0.80 | 4.03 | 14.29 |
| 3 | 0.83 | 2.87 | 3.48 | 2.14 | 34.81 | 1.77 | 6.96 | 25.96 |
| 4 | 0.71 | 1.69 | 2.39 | 1.65 | 18.48 | 0.89 | 5.28 | 18.00 |
| 5 | 0.89 | 1.57 | 1.76 | 2.76 | 25.12 | 1.66 | 5.58 | 44.23 |
| 6 | 0.70 | 1.54 | 2.18 | 2.31 | 24.98 | 1.33 | 5.55 | 37.54 |
| 7 | 0.81 | 1.45 | 1.79 | 0.91 | 44.28 | 1.94 | 8.20 | 27.80 |
| Mean | 0.80 | 1.66 | 2.09 | 1.73 | 27.13 | 1.34 | 5.67 | 27.49 |
| ±SEM | 0.04 | 0.21 | 0.26 | 0.27 | 3.48 | 0.17 | 0.56 | 3.95 |

Appendix 13 (contd.)

| Rat | Kidneys | | | | Heart | | | | Lungs | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Wt (g) | blood flow | | % CO | Wt (g) | blood flow | | % CO | Wt (g) | blood flow | | % CO |
| | | $ml.min^{-1}$ | $ml.min^{-1}.g^{-1}$ | | | $ml.min^{-1}$ | $ml.min^{-1}.g^{-1}$ | | | $ml.min^{-1}$ | $ml.min^{-1}g^{-1}$ | |
| 1 | 3.84 | 16.71 | 4.35 | 19.06 | 1.57 | 5.46 | 3.48 | 6.23 | 2.00 | 1.17 | 0.59 | 1.33 |
| 2 | 4.26 | 13.21 | 3.10 | 9.10 | 1.68 | 42.37 | 25.18 | 29.18 | 2.07 | 9.70 | 4.68 | 6.68 |
| 3 | 3.54 | 21.95 | 6.20 | 16.37 | 1.42 | 8.94 | 6.30 | 6.67 | 2.02 | 1.98 | 0.98 | 1.47 |
| 4 | 3.26 | 13.61 | 4.17 | 13.26 | 1.56 | 24.58 | 15.71 | 23.94 | 1.64 | 1.71 | 1.05 | 1.67 |
| 5 | 3.44 | 10.66 | 3.10 | 18.77 | 1.25 | 4.07 | 3.25 | 7.16 | 2.60 | 0.96 | 0.37 | 1.69 |
| 6 | 3.69 | 13.27 | 3.60 | 19.96 | 1.25 | 4.32 | 3.47 | 6.50 | 3.55 | 0.73 | 0.20 | 1.09 |
| 7 | 3.87 | 24.40 | 6.30 | 15.32 | 1.71 | 10.65 | 6.24 | 6.69 | 2.15 | 1.48 | 0.69 | 0.93 |
| Mean | 3.70 | 16.26 | 4.40 | 15.98 | 1.49 | 14.34 | 9.09 | 12.34 | 2.29 | 2.53 | 1.22 | 2.12 |
| ±SEM | 0.12 | 1.92 | 0.51 | 1.45 | 0.07 | 5.38 | 3.15 | 3.72 | 0.23 | 1.20 | 0.59 | 0.77 |

Appendix 14 Cardiac output and regional blood flow in corn oil treated rats.

| Rat no | Body weight (g) | Mean arterial pressure (mm.Hg) | Cardiac output $ml.min^{-1}$ | Liver | | | | | GI Tract and Pancreas | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Wt (g) | blood flow | | | % CO | Wt (g) | blood flow | | % CO |
| | | | | | $ml.min^{-1}$ | $ml.min^{-1}.g^{-1}$ | $g.100g,bw^{-1}$ | | | $ml.min^{-1}$ | $ml.min^{-1}.g^{-1}$ | |
| 1 | 425 | 112.50 | 110.51 | 15.02 | 5.35 | 0.36 | 3.53 | 4.84 | 11.90 | 13.42 | 1.13 | 12.14 |
| 2 | 460 | 125.05 | 72.81 | 17.13 | 3.35 | 0.20 | 3.69 | 4.60 | 12.43 | 10.83 | 0.87 | 14.87 |
| 3 | 450 | 122.00 | 132.38 | 14.07 | 4.21 | 0.30 | 3.13 | 3.18 | 12.60 | 11.49 | 0.91 | 8.68 |
| 4 | 345 | 112.50 | 62.24 | 11.58 | 3.11 | 0.27 | 3.36 | 4.99 | 12.83 | 8.34 | 0.65 | 13.39 |
| Mean | 395 | 118.01 | 94.48 | 14.45 | 4.00 | 0.28 | 3.43 | 4.40 | 12.44 | 11.02 | 0.89 | 12.27 |
| ±SEM | 50.54 | 3.24 | 16.33 | 1.15 | 0.51 | 0.03 | 0.12 | 0.91 | 0.20 | 1.05 | 0.10 | 1.32 |

Appendix 14 (contd.)

| Rat | Spleen | | | | Hepatosplanchnic | | | |
|---|---|---|---|---|---|---|---|---|
| | Wt (g) | blood flow | | % CO | blood flow | | | % CO |
| | | $ml.min^{-1}$ | $ml.min^{-1}.g^{-1}$ | | $ml.min^{-1}$ | $ml.min^{-1}.g^{-1}$ | $ml.min^{-1}$ $100g,bw^{-1}$ | |
| 1 | 0.80 | 2.50 | 3.14 | 2.26 | 21.27 | 1.42 | 5.00 | 19.25 |
| 2 | 0.69 | 1.08 | 1.56 | 1.49 | 15.26 | 0.89 | 3.32 | 20.96 |
| 3. | 0.81 | 2.87 | 3.54 | 2.17 | 18.57 | 1.32 | 4.13 | 14.03 |
| 4 | 0.59 | 0.89 | 1.51 | 1.43 | 12.34 | 1.06 | 3.57 | 19.83 |
| Mean | 0.72 | 1.83 | 2.44 | 1.84 | 16.86 | 1.17 | 4.00 | 18.51 |
| ±SEM | 0.05 | 0.50 | 0.53 | 0.22 | 1.94 | 0.12 | 0.37 | 1.54 |

Appendix 14 (contd.)

| Rat | Kidneys | | | | Heart | | | | Lungs | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Wt (g) | blood flow | | % CO | Wt (g) | blood flow | | % CO | Wt (g) | blood flow | | % CO |
| | | $ml.min^{-1}$ | $ml.min^{-1}.g^{-1}$ | | | $ml.min^{-1}$ | $ml.min^{-1}.g^{-1}$ | | | $ml.min^{-1}$ | $ml.min^{-1}g^{-1}$ | |
| 1 | 2.89 | 18.39 | 6.36 | 16.64 | 1.45 | 17.01 | 11.71 | 15.39 | 1.56 | 1.45 | 0.93 | 1.31 |
| 2 | 3.48 | 12.25 | 3.52 | 16.82 | 1.58 | 5.22 | 3.30 | 7.16 | 2.05 | 0.96 | 0.47 | 1.31 |
| 3 | 3.39 | 13.29 | 3.92 | 10.04 | 1.72 | 4.65 | 2.71 | 3.50 | 1.64 | 1.27 | 0.77 | 0.96 |
| 4 | 2.05 | 6.46 | 3.15 | 10.40 | 0.89 | 2.50 | 2.83 | 4.03 | 1.71 | 0.82 | 0.48 | 1.32 |
| Mean | 2.95 | 12.59 | 4.23 | 13.47 | 1.41 | 7.34 | 5.14 | 7.52 | 1.74 | 1.12 | 0.66 | 1.22 |
| ±SEM | 0.33 | 2.45 | 0.72 | 1.88 | 0.18 | 3.27 | 2.19 | 2.74 | 0.11 | 0.14 | 0.11 | 0.09 |

Appendix 15 Cardiac output and regional blood flow in 3,4 benzpyrene treated rats.

| Rat no | Body weight (g) | Mean arterial pressure (mm.Hg) | Cardiac output $ml.min^{-1}$ | Liver | | | | | GI Tract and Pancreas | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Wt (g) | blood flow | | | % CO | Wt (g) | blood flow | | % CO |
| | | | | | $ml.min^{-1}$ | $ml.min^{-1}.g^{-1}$ | $g.100g,bw^{-1}$ | | | $ml.min^{-1}$ | $ml.min^{-1}.g^{-1}$ | |
| 1 | 480 | 107.5 | 84.94 | 18.04 | 6.32 | 0.35 | 3.75 | 7.45 | 14.37 | 10.41 | 0.72 | 12.26 |
| 2 | 450 | 100 | 81.21 | 15.62 | 2.92 | 0.19 | 3.47 | 3.40 | 10.97 | 12.40 | 1.13 | 15.27 |
| 3 | 440 | 105 | 102.88 | 12.35 | 6.35 | 0.51 | 2.81 | 6.17 | 10.56 | 10.61 | 1.00 | 10.31 |
| 4 | 300 | 117.5 | 64.82 | 14.16 | 2.59 | 0.18 | 4.88 | 4.00 | 17.28 | 13.72 | 0.79 | 21.17 |
| 5 | 490 | 120 | 79.01 | 17.20 | 4.10 | 0.24 | 3.51 | 5.19 | 15.32 | 10.30 | 0.67 | 13.04 |
| 6 | 490 | 130 | 79.18 | 17.53 | 3.33 | 0.19 | 3.58 | 4.20 | 11.23 | 14.67 | 1.31 | 18.53 |
| Mean | 441.67 | 113.33 | 82.01 | 15.82 | 4.27 | 0.28 | 3.67 | 5.07 | 13.29 | 12.02 | 0.94 | 15.10 |
| ±SEM | 29.60 | 3.52 | 3.89 | 0.90 | 0.53 | 0.05 | 0.27 | 0.62 | 1.13 | 0.76 | 0.10 | 1.67 |

Appendix 15 (contd.)

| Rat | Spleen | | | | Hepatosplanchnic | | | |
|---|---|---|---|---|---|---|---|---|
| | Wt (g) | blood flow | | % CO | blood flow | | | % CO |
| | | $ml.min^{-1}$ | $ml.min^{-1}.g^{-1}$ | | $ml.min^{-1}$ | $ml.min^{-1}.g^{-1}$ | $ml.min^{-1}\ 100g,bw^{-1}$ | |
| 1 | 0.72 | 1.87 | 2.61 | 2.20 | 18.60 | 1.03 | 3.88 | 21.90 |
| 2 | 0.79 | 1.21 | 1.54 | 1.49 | 16.53 | 1.06 | 3.67 | 20.35 |
| 3 | 0.83 | 2.35 | 2.84 | 2.28 | 19.31 | 1.56 | 4.39 | 18.77 |
| 4 | 0.44 | 0.89 | 2.02 | 1.37 | 17.20 | 1.22 | 5.73 | 26.53 |
| 5 | 0.78 | 2.22 | 2.85 | 2.81 | 16.62 | 0.96 | 3.39 | 21.03 |
| 6 | 0.70 | 1.18 | 1.68 | 1.49 | 19.18 | 1.09 | 3.91 | 24.22 |
| Mean | 0.71 | 1.62 | 2.26 | 1.94 | 17.91 | 1.15 | 4.16 | 22.13 |
| ±SEM | 0.06 | 0.25 | 0.24 | 0.23 | 0.52 | 0.09 | 0.34 | 1.15 |

Appendix 15 (contd.)

| Rat | Kidneys | | | | Heart | | | | Lungs | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Wt (g) | blood flow | | % CO | Wt (g) | blood flow | | % CO | Wt (g) | blood flow | | % CO |
| | | $ml.min^{-1}$ | $ml.min^{-1}.g^{-1}$ | | | $ml.min^{-1}$ | $ml.min^{-1}.g^{-1}$ | | | $ml.min^{-1}$ | $ml.min^{-1}g^{-1}$ | |
| 1 | 3.58 | 19.43 | 5.43 | 22.87 | 1.36 | 3.35 | 2.46 | 3.94 | 1.46 | 0.36 | 0.25 | 0.42 |
| 2 | 3.50 | 17.88 | 5.11 | 22.02 | 1.53 | 4.26 | 2.79 | 5.25 | 1.81 | 0.89 | 0.49 | 1.20 |
| 3 | 3.00 | 11.49 | 3.83 | 11.17 | 1.40 | 6.04 | 4.32 | 5.87 | 1.62 | 2.05 | 1.27 | 2.00 |
| 4 | 2.83 | 8.43 | 2.98 | 13.54 | 1.28 | 3.13 | 2.44 | 4.83 | 2.26 | 4.37 | 1.93 | 6.75 |
| 5 | 4.00 | 14.02 | 3.50 | 17.74 | 2.06 | 2.49 | 1.21 | 3.15 | 1.58 | 1.00 | 0.63 | 1.26 |
| 6 | 4.26 | 16.72 | 3.92 | 21.12 | 1.68 | 4.62 | 2.74 | 5.83 | 1.81 | 1.52 | 0.84 | 1.92 |
| Mean | 3.53 | 14.66 | 4.13 | 18.08 | 1.55 | 3.98 | 2.66 | 4.81 | 1.76 | 1.70 | 0.90 | 2.26 |
| ±SEM | 0.22 | 1.70 | 0.39 | 1.97 | 0.12 | 0.52 | 0.41 | 0.44 | 0.11 | 0.58 | 0.25 | 0.93 |

Appendix 16 Cardiac output and regional blood flow in sotalol treated rats

| Rat no | Body weight (g) | Mean arterial pressure (mm.Hg) | Cardiac output $ml.min^{-1}$ | Liver | | | | | GI Tract and Pancreas | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Wt (g) | blood flow | | | % CO | Wt (g) | blood flow | | % CO |
| | | | | | $ml.min^{-1}$ | $ml.min^{-1}.g^{-1}$ | $g.100g,bw^{-1}$ | | | $ml.min^{-1}$ | $ml.min^{-1}.g^{-1}$ | |
| 1 | 415 | 75 | 153.04 | 16.31 | 3.43 | 0.21 | 3.93 | 5.40 | 14.03 | 7.38 | 0.53 | 11.62 |
| 2 | 370 | 65 | 51.13 | 13.93 | 2.28 | 0.16 | 3.76 | 4.45 | 13.21 | 9.03 | 0.68 | 17.66 |
| 3 | 370 | 87.50 | 59.20 | 13.89 | 2.33 | 0.17 | 3.75 | 3.93 | 13.71 | 8.85 | 0.64 | 14.95 |
| 4 | 375 | 90 | 56.03 | 13.03 | 1.78 | 0.14 | 3.47 | 2.35 | 11.65 | 12.99 | 1.11 | 17.09 |
| 5 | 245 | 112.5 | 43.24 | 11.58 | 0.48 | 0.04 | 4.73 | 1.11 | 14.83 | 8.48 | 0.57 | 19.62 |
| 6 | 390 | 72.5 | 38.75 | 12.91 | 1.18 | 0.09 | 3.31 | 3.04 | 8.54 | 5.93 | 0.69 | 15.30 |
| 7 | 390 | 80. | 41.69 | 12.47 | 0.00 | 0.16 | 3.20 | 4.77 | 10.87 | 5.01 | 0.46 | 12.01 |
| Mean | 365 | 83.21 | 50.51 | 13.44 | 1.92 | 0.14 | 3.73 | 3.58 | 12.40 | 8.24 | 0.67 | 15.46 |
| ±SEM | 20.64 | 5.87 | 3.60 | 0.57 | 0.35 | 0.02 | 0.19 | 0.57 | 0.83 | 0.98 | 0.08 | 1.11 |

Appendix 16 (contd.

| Rat | Spleen | | | | Hepatosplanchnic | | | |
|---|---|---|---|---|---|---|---|---|
| | Wt (g) | blood flow | | % CO | blood flow | | | % CO |
| | | $ml.min^{-1}$ | $ml.min^{-1}.g^{-1}$ | | $ml.min^{-1}$ | $ml.min^{-1}.g^{-1}$ | $ml.min^{-1}100g,bw^{-1}$ | |
| 1 | 0.83 | 1.30 | 1.56 | 2.05 | 12.11 | 0.74 | 2.92 | 19.07 |
| 2 | 0.74 | 1.04 | 1.41 | 2.03 | 12.35 | 0.89 | 3.63 | 24.15 |
| 3 | 0.92 | 1.30 | 1.41 | 2.19 | 12.48 | 0.90 | 3.37 | 21.08 |
| 4 | 0.91 | 1.86 | 2.05 | 2.45 | 16.63 | 1.27 | 4.43 | 21.88 |
| 5 | 0.59 | 0.05 | 0.08 | 0.11 | 9.01 | 0.78 | 3.68 | 20.84 |
| 6 | 0.95 | 0.43 | 0.45 | 1.11 | 7.54 | 0.58 | 1.93 | 19.45 |
| 7 | 0.90 | 0.87 | 0.97 | 2.09 | 7.87 | 0.63 | 2.02 | 18.87 |
| Mean | 0.83 | 0.98 | 1.13 | 1.72 | 11.14 | 0.83 | 3.14 | 20.76 |
| ±SEM | 0.05 | 0.23 | 0.26 | 0.31 | 1.22 | 0.09 | 0.34 | 0.71 |

Appendix 16 (contd.)

| Rat | Kidneys | | | | Heart | | | | Lungs | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Wt (g) | blood flow | | % CO | Wt (g) | blood flow | | % CO | Wt (g) | blood flow | | % CO |
| | | $ml.min^{-1}$ | $ml.min^{-1}.g^{-1}$ | | | $ml.min^{-1}$ | $ml.min^{-1}.g^{-1}$ | | | $ml.min^{-1}$ | $ml.min^{-1}g^{-1}$ | |
| 1 | 3.39 | 8.25 | 2.43 | 12.99 | 1.41 | 4.14 | 2.94 | 6.52 | 1.85 | 4.48 | 2.42 | 7.05 |
| 2 | 3.34 | 10.23 | 3.06 | 20.03 | 1.06 | 2.20 | 2.07 | 4.30 | 1.85 | 0.36 | 0.20 | 0.71 |
| 3 | 3.08 | 11.75 | 3.81 | 19.85 | 1.46 | 2.81 | 1.93 | 4.75 | 2.19 | 1.07 | 0.49 | 1.81 |
| 4 | 3.02 | 16.36 | 5.42 | 21.51 | 1.58 | 3.77 | 2.36 | 4.95 | 2.14 | 0.60 | 0.28 | 0.79 |
| 5 | 2.15 | 6.07 | 2.82 | 14.04 | 0.89 | 2.30 | 2.60 | 5.33 | 1.71 | 0.62 | 0.36 | 1.44 |
| 6 | 3.28 | 8.65 | 2.64 | 22.33 | 1.26 | 1.46 | 1.16 | 3.76 | 3.58 | 0.17 | 0.05 | 0.44 |
| 7 | 3.22 | 8.77 | 2.72 | 21.04 | 1.48 | 1.73 | 1.17 | 4.15 | 3.18 | 0.20 | 0.06 | 0.48 |
| Mean | 3.07 | 10.01 | 3.27 | 18.83 | 1.30 | 2.63 | 2.03 | 4.82 | 2.36 | 1.07 | 0.55 | 1.82 |
| ±SEM | 0.16 | 1.25 | 0.39 | 1.41 | 0.09 | 0.38 | 0.26 | 0.34 | 0.27 | 0.58 | 0.32 | 0.89 |

Appendix 17 Cardiac output and regional blood flow in rats receiving lignocaine 5 $mg.kg^{-1}$ intravenously.

| Rat no | Body weight (g) | Mean arterial pressure (mm.Hg) | Cardiac output $ml.min^{-1}$ | Liver | | | | | GI Tract and Pancreas | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Wt (g) | blood flow | | | % CO | Wt (g) | blood flow | | % CO |
| | | | | | $ml.min^{-1}$ | $ml.min^{-1}.g^{-1}$ | $g.100g,bw^{-1}$ | | | $ml.min^{-1}$ | $ml.min^{-1}.g^{-1}$ | |
| 1 | 350 | 107.5 | 181.03 | 13.30 | 2.80 | 0.21 | 3.80 | 3.45 | 15.96 | 12.00 | 0.75 | 14.81 |
| 2 | 490 | 110.0 | 120.21 | 15.63 | 3.48 | 0.22 | 3.19 | 2.89 | 10.59 | 17.40 | 1.64 | 14.47 |
| 3 | 500 | 125.0 | 96.40 | 16.92 | 3.90 | 0.23 | 3.38 | 4.04 | 9.67 | 8.78 | 0.91 | 9.11 |
| 4 | 490 | 110.0 | 89.26 | 16.24 | 3.30 | 0.20 | 3.84 | 3.70 | 11.86 | 13.72 | 1.16 | 15.37 |
| Mean | 457.5 | 113.12 | 121.72 | 15.52 | 3.37 | 0.21 | 3.55 | 3.52 | 12.02 | 12.97 | 1.11 | 13.44 |
| ±SEM | 35.91 | 4.00 | 20.85 | 0.79 | 0.23 | 0.006 | 0.16 | 0.24 | 1.39 | 1.79 | 0.19 | 1.45 |

Appendix 17 (contd.)

| Rat | Spleen | | | | Hepatosplanchnic | | | |
|---|---|---|---|---|---|---|---|---|
| | Wt (g) | blood flow | | % CO | blood flow | | | % CO |
| | | $ml.min^{-1}$ | $ml.min^{-1}.g^{-1}$ | | $ml.min^{-1}$ | $ml.min^{-1}.g^{-1}$ | $ml.min^{-1}$ $100g,bw^{-1}$ | |
| 1 | 0.99 | 1.48 | 1.49 | 1.83 | 16.28 | 1.22 | 4.65 | 20.09 |
| 2 | 0.73 | 2.50 | 3.41 | 2.08 | 23.38 | 1.50 | 4.77 | 19.45 |
| 3 | 0.79 | 1.51 | 1.92 | 1.57 | 14.19 | 0.84 | 2.84 | 14.70 |
| 4. | 1.03 | 1.80 | 1.75 | 2.02 | 18.82 | 1.15 | 3.84 | 21.08 |
| Mean | 0.88 | 1.82 | 2.14 | 1.87 | 18.17 | 1.18 | 4.02 | 18.83 |
| ±SEM | 0.07 | 0.24 | 0.43 | 0.11 | 1.98 | 0.13 | 0.44 | 1.41 |

Appendix 17 (contd.)

| Rat | Kidneys | | | | Heart | | | | Lungs | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Wt (g) | blood flow | | % CO | Wt (g) | blood flow | | % CO | Wt (g) | blood flow | | % CO |
| | | $ml.min^{-1}$ | $ml.min^{-1}.g^{-1}$ | | | $ml.min^{-1}$ | $ml.min^{-1}.g^{-1}$ | | | $ml.min^{-1}$ | $ml.min^{-1}g^{-1}$ | |
| 1 | 3.02 | 13.35 | 4.42 | 16.47 | 1.15 | 2.67 | 2.32 | 3.29 | 1.54 | 1.53 | 1.00 | 1.89 |
| 2 | 3.48 | 16.68 | 4.79 | 13.87 | 1.40 | 5.20 | 3.71 | 4.32 | 2.00 | 1.56 | 0.79 | 1.30 |
| 3 | 3.55 | 12.90 | 3.63 | 13.88 | 1.53 | 8.02 | 5.23 | 8.32 | 2.63 | 1.98 | 0.75 | 2.05 |
| 4 | 3.18 | 12.16 | 3.83 | 13.12 | 1.41 | 3.50 | 2.48 | 3.92 | 2.00 | 3.60 | 1.80 | 4.03 |
| Mean | 3.31 | 13.77 | 4.17 | 14.46 | 1.37 | 4.85 | 3.43 | 4.96 | 2.04 | 2.17 | 1.08 | 2.32 |
| ±SEM | 0.12 | 1.00 | 0.27 | 0.67 | 0.08 | 1.18 | 0.67 | 1.14 | 0.22 | 0.49 | 0.24 | 0.59 |

Appendix 18 Cardiac output and regional blood flow in rats receiving tocainide 35 mg.kg$^{-1}$ intravenously

| Rat no | Body weight (g) | Mean arterial pressure (mm.Hg) | Cardiac output ml.min$^{-1}$ | Liver | | | | | GI Tract and Pancreas | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Wt (g) | blood flow | | | % CO | Wt (g) | blood flow | | % CO |
| | | | | | ml.min$^{-1}$ | ml.min$^{-1}$. g$^{-1}$ | g.100g,bw$^{-1}$ | | | ml.min$^{-1}$ | ml.min$^{-1}$. g$^{-1}$ | |
| 1 | 510 | 117.5 | 125.40 | 16.28 | 4.38 | 0.27 | 3.16 | 3.49 | 16.56 | 13.83 | 0.85 | 11.03 |
| 2 | 570 | 117.5 | 156.28 | 18.95 | 2.71 | 0.14 | 3.32 | 1.73 | 12.02 | 21.59 | 1.80 | 13.81 |
| 3 | 475 | 140 | 78.66 | 16.07 | 2.26 | 0.14 | 3.38 | 2.87 | 15.30 | 13.86 | 0.90 | 17.58 |
| 4 | 480 | 107.5 | 75.94 | 15.61 | 2.19 | 0.14 | 3.25 | 2.88 | 19.43 | 15.02 | 0.77 | 18.16 |
| Mean | 508.75 | 120.62 | 10.07 | 16.73 | 2.88 | 0.17 | 3.28 | 2.74 | 15.83 | 16.07 | 1.08 | 15.55 |
| ±SEM | 21.83 | 6.87 | 19.40 | 0.75 | 0.51 | 0.03 | 0.05 | 0.37 | 1.53 | 1.86 | 0.24 | 1.95 |

Appendix 18 (contd.)

| Rat | Spleen | | | | Hepatosplanchnic | | | |
|---|---|---|---|---|---|---|---|---|
| | Wt (g) | blood flow | | % CO | blood flow | | | % CO |
| | | $ml.min^{-1}$ | $ml.min^{-1}.g^{-1}$ | | $ml.min^{-1}$ | $ml.min^{-1}.g^{-1}$ | $ml.min^{-1}$ $100g,bw^{-1}$ | |
| 1 | 0.92 | 2.41 | 2.62 | 1.92 | 20.62 | 1.27 | 4.04 | 16.44 |
| 2 | 0.85 | 2.39 | 2.79 | 1.53 | 26.69 | 1.41 | 4.68 | 17.08 |
| 3 | 0.55 | 1.66 | 3.02 | 2.11 | 17.78 | 0.98 | 3.74 | 22.60 |
| 4 | 0.72 | 0.95 | 1.32 | 1.25 | 18.16 | 1.16 | 3.78 | 23.91 |
| Mean | 0.76 | 1.84 | 2.44 | 1.70 | 20.81 | 1.20 | 4.06 | 20.01 |
| ±SEM | 0.08 | 0.35 | 0.38 | 0.19 | 2.06 | 0.09 | 0.22 | 1.90 |

Appendix 18 (contd.)

| Rat | Kidneys | | | | Heart | | | | Lungs | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Wt (g) | blood flow | | % CO | Wt (g) | blood flow | | % CO | Wt (g) | blood flow | | % CO |
| | | $ml.min^{-1}$ | $ml.min^{-1}.g^{-1}$ | | | $ml.min^{-1}$ | $ml.min^{-1}.g^{-1}$ | | | $ml.min^{-1}$ | $ml.min^{-1}g^{-1}$ | |
| 1 | 3.69 | 15.87 | 4.29 | 12.64 | 2.12 | 6.47 | 3.04 | 5.16 | 2.23 | 2.10 | 0.94 | 1.67 |
| 2 | 3.86 | 30.68 | 7.94 | 19.63 | 2.14 | 6.57 | 3.07 | 4.20 | 2.23 | 1.05 | 0.47 | 0.67 |
| 3 | 3.07 | 12.29 | 4.00 | 15.62 | 1.20 | 2.88 | 2.39 | 3.66 | 1.45 | 1.56 | 1.07 | 1.24 |
| 4 | 4.20 | 19.30 | 4.59 | 25.41 | 1.36 | 2.69 | 1.97 | 3.54 | 3.35 | 0.77 | 0.23 | 1.01 |
| Mean | 3.70 | 19.53 | 5.20 | 18.32 | 1.70 | 4.65 | 2.62 | 4.14 | 2.31 | 1.37 | 0.68 | 1.14 |
| ±SEM | 0.24 | 3.98 | 0.92 | 2.76 | 0.25 | 1.08 | 0.27 | 0.37 | 0.39 | 0.29 | 0.20 | 0.21 |

Appendix 19 Cardiac output and regional blood flow in phenobarbitone treated rats which received tocainide 35 $mg.kg^{-1}$ intravenously

| Rat no | Body weight (g) | Mean arterial pressure (mm.Hg) | Cardiac output $ml.min^{-1}$ | Liver | | | | | GI Tract and Pancreas | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Wt (g) | blood flow | | | % CO | Wt (g) | blood flow | | % CO |
| | | | | | $ml.min^{-1}$ | $ml.min^{-1}.g^{-1}$ | $g.100g,bw^{-1}$ | | | $ml.min^{-1}$ | $ml.min^{-1}.g^{-1}$ | |
| 1 | 300 | 137.5 | 68.79 | 13.36 | 3.28 | 0.24 | 4.45 | 4.77 | 19.10 | 18.16 | 0.95 | 26.40 |
| 2 | 300 | 117.5 | 64.82 | 14.16 | 2.59 | 0.18 | 4.72 | 4.00 | 17.28 | 13.72 | 0.79 | 21.17 |
| 3 | 550 | 115 | 99.48 | 24.05 | 6.86 | 0.28 | 4.37 | 6.89 | 23.78 | 21.24 | 0.89 | 21.35 |
| 4 | 425 | 117.5 | 81.65 | 17.79 | 3.73 | 0.21 | 4.19 | 4.57 | 11.94 | 14.52 | 1.22 | 17.78 |
| Mean | 393.75 | 121.87 | 78.68 | 17.34 | 4.11 | 0.23 | 4.43 | 5.06 | 18.02 | 16.91 | 0.96 | 21.67 |
| ±SEM | 59.84 | 5.24 | 7.81 | 2.43 | 0.94 | 0.02 | 0.11 | 0.63 | 2.45 | 1.74 | 0.09 | 1.78 |

Appendix 19 (contd.)

| Rat | Spleen | | | | Hepatosplanchnic | | | |
|---|---|---|---|---|---|---|---|---|
| | Wt (g) | blood flow | | % CO | blood flow | | | % CO |
| | | $ml.min^{-1}$ | $ml.min^{-1}.g^{-1}$ | | $ml.min^{-1}$ | $ml.min^{-1}.g^{-1}$ | $ml.min^{-1}\ 100g,bw^{-1}$ | |
| 1 | 0.93 | 1.64 | 1.76 | 2.38 | 23.08 | 1.73 | 7.69 | 33.55 |
| 2 | 0.44 | 0.89 | 2.02 | 1.37 | 17.20 | 1.22 | 5.73 | 26.53 |
| 3 | 0.90 | 1.56 | 1.73 | 1.57 | 29.66 | 1.23 | 5.39 | 29.81 |
| 4 | 0.87 | 1.35 | 1.54 | 1.65 | 19.60 | 1.10 | 4.61 | 24.00 |
| Mean | 0.78 | 1.36 | 1.76 | 1.74 | 22.38 | 1.32 | 5.85 | 28.47 |
| ±SEM | 0.11 | 0.17 | 0.10 | 0.22 | 2.71 | 0.14 | 0.65 | 2.07 |

Appendix 19 (contd.)

| Rat | Kidneys | | | | Heart | | | | Lungs | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Wt (g) | blood flow | | % CO | Wt (g) | blood flow | | % CO | Wt (g) | blood flow | | % CO |
| | | $ml.min^{-1}$ | $ml.min^{-1}.g^{-1}$ | | | $ml.min^{-1}$ | $ml.min^{-1}.g^{-1}$ | | | $ml.min^{-1}$ | $ml.min^{-1}g^{-1}$ | |
| 1 | 2.54 | 7.31 | 2.88 | 10.62 | 1.10 | 4.20 | 3.82 | 6.10 | 1.41 | 1.01 | 2.72 | 1.47 |
| 2 | 2.83 | 8.43 | 2.97 | 13.00 | 1.28 | 3.13 | 2.44 | 4.83 | 2.26 | 4.37 | 1.93 | 6.75 |
| 3 | 4.37 | 20.94 | 4.79 | 21.05 | 1.67 | 3.45 | 2.07 | 2.08 | 2.98 | 2.76 | 0.93 | 2.77 |
| 4 | 3.39 | 15.19 | 4.48 | 18.60 | 1.29 | 2.54 | 1.97 | 3.11 | 2.49 | 0.95 | 0.38 | 1.16 |
| Mean | 3.28 | 12.97 | 3.78 | 15.82 | 1.33 | 3.33 | 2.57 | 4.03 | 2.28 | 2.20 | 1.49 | 3.04 |
| ±SEM | 0.40 | 3.18 | 0.50 | 2.42 | 0.12 | 0.34 | 0.43 | 0.89 | 0.33 | 0.80 | 0.52 | 1.28 |

Appendix 20

Pharmacokinetic calculations

Lignocaine and tocainide blood concentration-time data after i.v. administration were fitted to prescribed functions using the non-linear least squares regression analysis programme "NONLIN" (Metzler, 1969). Preliminary analysis indicated that it was adequate to fit the lignocaine and tocainide I.V. bolus dose data by the sum of two exponentials.

The following parameters were calculated from the coefficients and exponents ($\alpha$ referring to the fast and $\beta$ to the slow exponent) of the biexponential equation describing the lignocaine and tocainide intravenous data

$k_{12}$, $k_{21}$ and $k_e$

Vp

$t_{\frac{1}{2}\alpha}$, $t_{\frac{1}{2}\beta}$

AUC

CL

Distribution volumes, $VD_{extrap}$, $Vd_{area}$ and $VD_{ss}$ were calculated from the following manners:

$$Vd_{extrap} = \frac{V_p \cdot (\alpha-\beta)}{k_{21}-\beta} = \frac{D_{i.v.}}{B} \qquad \text{eq. (14)}$$

$$Vd_{area} = \frac{Dose}{\beta \cdot area} \qquad \text{eq. (19)}$$

and

$$Vd_{ss} = \frac{k_{12} + k_{21}}{k_{21}} \cdot Vp \qquad \text{eq. (21)}$$

The blood concentration data of the drugs after single oral administration were fitted by least squares linear regression analysis to a monoexponential equation. The estimated of $k_{ab}$, $k_d$ and $t_{\frac{1}{2}}$ were obtained graphically from semilogarithmic blood concentration curves by the method described by Gibaldi (1975) and Wagner (1975) (see Chapter I).

The areas under the blood concentration-time curve after oral administration were calculated according to the trapezoidal rule (Notari, 1975) and the infinite part of the curve was calculated as $C^*/k_d$ ($C^*$ = last measurable concentration, $k_d$ = overall elimination rate constant).

Systemic availability of orally administered lignocaine and tocainide was calculated according to the principles discussed previously (Chapter I) and shown in eq. (23).

$$\text{Systemic availability} = \frac{AUC_{oral}}{AUC_{i.v.}} \qquad \text{eq. (23)}$$

Mean renal clearance of i.v. tocainide was calculated from the blood level and urinary excretion data by the following relatinship

$$\text{Mean renal clearance} = \frac{\text{Total amount excreted in urine}}{\text{Total area under blood concentration-time curve}} \qquad \text{eq. (37)}$$

The total body clearance (mean blood clearance) after i.v. and oral administration was obtained from eq. (15).

$$CL = \frac{Dose}{AUC} \qquad \text{eq. (15)}$$

The difference between total body and renal clearance was designated as the "nonrenal clearance".

Appendix 21 The nonlinear least square regression analysis

programme "NONLIN"

```
TRN4 000530  SOURCE PROGRAM

  1 C       INTRAVENOUS DOSE...TWO-COMPARTMENT MODEL
  2         SUBROUTINE DFUNC(F,P,CON,VAL,X,I,J,ISPEC,XVEC,Y,W,NOBS)
  3         IMPLICIT REAL*8(A-H,O-Z)
  4         DIMENSION ISPEC(1),NOBS(1)
  5         DIMENSION P(1),CON(1),VAL(1),XVEC(1),Y(1),W(1)
  6         W(1)=0.
  7         DOSE=CON(1)
  8         SUM=P(1)+P(2)+P(3)
  9         PROD=4.*P(2)*P(3)
 10         ALPHA=(SUM+SQRT(SUM*SUM-PROD))/2.
 11         BETA =(SUM-SQRT(SUM*SUM-PROD))/2.
 12         A=DOSE/P(4)*(P(2)-ALPHA)/(BETA-ALPHA)
 13         B=DOSE/P(4)*(BETA -P(2))/(BETA-ALPHA)
 14         F=A*EXP(-ALPHA*X)+B*EXP(-BETA*X)
 15         IF(ISPEC(8).NE.0)RETURN
 16         WRITE(6,1)CON(1),P(1),P(2),P(3),P(4)
 17       1 FORMAT(/' DOSE =',F12.6,' MICROGRAMS'/
 18        1' K12 =',F12.6,' K21 =',F12.6,' K13 =',F12.6,'  MIN-1'/
 19        2' VOLUME OF CENTRAL COMPARTMENT =',F12.6,'  MLS')
 20         T12A=0.693/ALPHA
 21         T12B=0.693/BETA
 22         AUC=A/ALPHA+B/BETA
 23         CL=DOSE/AUC
 24         WRITE(6,2)A,B,ALPHA,T12A,BETA,T12B,AUC,CL
 25       2 FORMAT(/' A =',F12.6,' B =',F12.6/
 26        1' ALPHA =' ,F12.6,5X,'T1/2 =',F12.6/
 27        2' BETA  =',F12.6,5X,'T1/2 =',F12.6/
 28        3         ' AUC =',F12.6,5X,'CL =',F12.6)
 29         NTIM=ISPEC(2)
 30         FACTOR=P(1)*      DOSE/(ALPHA-BETA)
 31         WRITE(6,3)
 32       3 FORMAT(//' TIME',6X,'DRUG CONCENTRATION IN',5X,'AMOUNT OF DRUG IN
 33        1,10X,'AMOUNT OF DRUG IN',10X,'TOTAL AMOUNT'/
 34        2          ' (MIN)',5X,'CENTRAL COMPARTMENT',7X,'CENTRAL COMPARTMENT
 35        3',8X,'PERIPHERAL COMPARTMENT',5X,'OF DRUG XC+XP'/
 36        4          11X,'(OBS; MICROGRAMS/ML)',6X,'(ESTIMATE; MICROGRAMS)',
 37        55X,'(ESTIMATE; MICROGRAMS)',6X,'(MICROGRAMS)')
 38         DO 4 N=2,NTIM
 39         XC=Y(N)*P(4)
 40         XX      =XVEC(N)
 41         XP=FACTOR*(EXP(-BETA*XX)-EXP(-ALPHA*XX))
 42         XT=XC+XP
 43       4 WRITE(6,5)XVEC(N),Y(N),XC,XP,XT
 44       5 FORMAT(' ',F5.1,12X,F7.3,19X,F10.3,17X,F10.3,13X,F10.3)
 45         RETURN
 46         END
```

Appendix 22

Phosphate buffer (pH 7.25)

Dissolve 9.2 g potassium dihydrogen phosphate ($KH_2PO_4$)

44.0 g dipotassium hydrogen phosphate ($K_2HPO_4.4H_2O$)

11.2 g potassium chloride (KCl);

in sufficient amount of de-ionised water to produce 1000 ml. Adjust pH to 7.25 with 0.2 N HCl or NaOH

Appendix 23

Glycerol phosphate buffer (pH 7.25)

Dissolve 9.2 g potassium dihydrogen phosphate ($KH_2PO_4$)

44.0 g dipotassium hydrogen phosphate ($K_2HPO_4$ $4H_2O$)

in sufficient amount of deionised water to produce 700 ml. Adjust pH to 7.25 with 0.2 N HCl or NaOH and then add 3.00 ml of glycerol to the mixture.

Appendix 24

Incubation mixture for p-nitroanisole O-demethylase activity assay.

Incubation mixture allowing for each sample:

0.25 ml 0.2 M Tris pH 7.2

0.03 ml 0.1 M $MgCl_2$

0.62 ml $H_2O$

0.3 mg NADH

0.3 mg NADPH

Titrate to pH 7.2 with 0.2 N NaOH if necessary.

Appendix 25

Citrate buffer (pH 5.6)

Dissolve 7.14 g citric acid;

44.1 g sodium citrate;

in 620 ml deionised water.

Adjust to pH 5.6 with 1 M citric acid solution.

Appendix 26

Nash reagent

Prepare fresh for each assay

3.75 g ammonium acetate;

0.05 ml acetylacetone;

to 50 ml with distilled water.

# BIBLIOGRAPHY

ADAM (1972)
Astra Report No. 423 W34.

ADJEPON-YAMOAH, K.K. and PRESCOTT, L.F. (1973).
Br. J. Pharmacol., 47, 672-673.

AHMAD, K. and MEOZIHRADSKY, F. (1971).
Res. Comm. Chem. Pathol., Pharmacol., 2, 6, 813-826.

ÅKERMAN, B., ASTRÖM, A., ROSS, S. and TELC, A. (1966).
Acta Pharmacol et toxicol., 24, 389-403.

APS, C., BELL, J.A., JENKINS, B.S., POOLE-WILSON, P.A. and REYNOLDS, F. (1975).
Br. Med. J., 1, 13-15.

ARCHIE, J.P., FIXLER, D.E., ULLYOT, D.J., HOFFMAN, J., UTLEY, J.R. and CARLSON, E.L. (1973).
J. Appl. Physiol., 35, 1, 148-154.

AUSTEN, W.G. and MORAN, J.M. (1965).
Am J. Cardiol., 16, 701-707.

VON BAHR, C., BORGA, O., FELLENIUS, E. and ROWLAND, M. (1973).
Pharmacology, 9, 177-186.

VON BAHR, C., HEDLUND, I., KARLEN, B., BÄCKSTRÖM, D. and GRASDALEN, H. (1977).
Acta pharmacol et toxicol., 41, 39-48.

BARTRUM, R.J., BERKOWITZ, D.M. and HOLLENBERG, N.K. (1974).
Inv. Radiology, 9, 126-132.

BECKETT, A.H., BOYES, R.N. and APPLETON, P.J. (1966).
J. Pharm. Pharmacol., 18, suppl. 765-815.

BECKETT, A.H., BOYES, R.N. and PARKER, J.B.R. (1965).
Anaesthesia, 20: 3, 294-298.

BELL, G.J., HILEY, C.R. and YATES, M.S. (1979).
Proc. of the B.P.S., July, 1977, 126-127.

BENET, L.Z. and RONFELD, R.A. (1969).
J. Pharm. Sci., 58: 5, 639-641.

BENNETT, P.N., BENDING, M.R., STEINER, J.A. and ROWLAND, M. (1978).
Pros. 7th Int. Cong. Pharmacol., Paris, 1978.
Eds. Boissiel, J.R. and Le Chat, P. Pergamon.

BENNETT, P.N., AARONS, L., BENDING, M.R., STEINER, J.A. and ROWLAND, M. (in press).

BENOWITZ, N., FORSYTH, R.P., MELMON, K.L. and ROWLAND, M. (1974a).
Clin. Pharmacol. Ther., 16: 1, 87-98.

BENOWITZ, N., FORSYTH, R.P., MELMON, K.L. and ROWLAND, M. (1974b).
Clin. Pharmacol. Ther., 16: 1, 99-109.

BINNION, P.F., MURTAGH, G., POLLOCK, A.M. and FLETCHER, E. (1969).
Brit. M. J., 3, 390-392.

BISCHOFF, K.B. and DEDRICK, R.L. (1968).
J. Pharm. Sci., 57 , 1346-1351.

BLUMER, J., STRONG, J.M. and ATKINSON, A.J. (1971).
J. Pharmacol. Exp. Ther., 180: L, 31-36.

BOYES, R.N., ADAMS, H.J. and DUCE, B.R. (1970).
J. Pharmacol. Exp Ther., 174: 1, 1-8.

BOYES, R.N., SCOTT, D.V., JEBSON, P.J., GODMAN, M.J. and JULIAN, D.G. (1971).
Clin. Pharmacol. Therap. 12, 105-116.

BRANCH, R.A., SHAND, D.G., WILKINSON, G.R. and NIES, A.S. (1973).
Clin. Pharmacol. Exp. Ther., 184: 2, 515-519.

BRANCH, R.A., SHAND, D.G., WILKINSON, G.R. and NIES, A.S. (1974).
J. Clin. Invest., 53, 1101-1107.

BRECK, G.D. and TRAGER, W.F. (1971).
Sci, 173, 544-546.

BRODIE, B.B., GILLETTE, J.R. and LA DU, B.N. (1958).
Ann. Rev. Biochem., 27, 427-454.

BULLOCK, K. and GRUNDY, J. (1955).
J. Pharm. Pharamcol., 7, 755-773.

BURNEY, R.G., DIFAZIO, C.A., PEACH, M.J., PETRIE, K.A. and SILVESTER, M.J. (1974).
Am. Heart. J., 88: 6, 765-769.

CAESAR, J., SHALDON, S., CHIANDUSSI, L., GUEVARA, L. and SHERLOCK, S. (1961).
Clin. Sci., 21, 43-57.

CARDEN, N.L. and STEINHAUS, J.E. (1956).
Circ. Res., 4, 680-683.

COLLINSWORTH, K. (1976).
West. J. Med., 124, 36.

COLLINSWORTH, K.A., STRONG, J.M., ATKINSON, A.J., WINKLE, R.A., PERLROTH, F. and HARRISON, D.C. (1975).
Clin. Pharmacol. Ther. 18: 1, 59-64.

COLTART, D.J., BERNDT, T.B., KERNOFF, R. and HARRISON, D.C. (1974).
Am. J. Cardiol., 34, 35-41.

CONN, H.L. (1955).
J. Clin. Invest., 34, 916-917.

CONNEY, A.H. (1967).
Pharmacol. Revs., 19: 3, 317-366.

CONNEY, A.H., DIVISON, C., GASTEL, R. and BURNS, J.J. (1960).
J. Pharmacol. Exp Ther., 130, 1-8.

CONNEY, A.H., GILLETTE, J.R., Inscoe, J.K. Trams, E.O. and Posner, H.S. (1959).
Science, 130, 1478-1479.

CONNEY, A.H., Miller, E.C. and MILLER J.A. (1957).
J. Biol. Chem., 228, 753-766.

CONWAY, W.D., MINATOYA, H., LANDS, A.M. and SHEKOSKY, J.M. (1968).
J. Pharm. Sci., 57, 1135-1141.

CULBERTSON, J.W., WILKINS, R.W., INGLEFINGER, F.J. and BRADLEY, S.E. (1951).
J. Clin. Invest., 30, 305-311.

CULLHED, I. (1969).
Acta. med. Scand., 186, 53-55.

CURRY, S.H., D'MELLO, A. and MOULD, G.P. (1971).
Br. J. Pharmacol., 42, 403-411.

DAVIS, L.D. and TEMTE, J.V. (1969).
Cir. Res., 24, 639-655.

DEACOCK, A.R. and SIMPSON, W.T. (1964).
Anaesthesia, 19: 2, 217-221.

DIFAZIO, C.A. (1975).
Int. Anaesthesiol. Clin., 13,: 4, 21-31.

DIFAZIO, C.A. and BROWN, R.E. (1972).
Anaesthesiology, 36: 3, 238-243.

DUCE, B.R., SMITH, E.R., BOYES, R.N. and BYRNES, E.W. (1973).
Pharmacologist, 15, 192.

DUCE, B.R. (1972).
Astra Report No. 423 W51.

DVORCHIK, B.H. and Vessel, E.S. (1978).
Clin. Pharmacol. Ther., 23: 6, 617-623.

DOBSON, E.L. and JONES, H.B. (1952).
Acta Med. Scand., 144, suppl. 273, 1-71.

EHRENBERG, L. (1948).
Acta. Chem. Scand. 2, 63-81.

ERIKSSON, E. and GRANBERG, P.O. (1965).
Acta Anaesth. Scandinav., Suppl. 16, 79-85.

ELVIN, A.T., KEENAGHAN, J.B., BYRNES, E.W., TENTHOREY, P.A., MCMASTER, P.D., TAKMAN, B.H., LALKA, D., MEYER, M.B. and RONFELD, R.A. (1980).
J. Pharm. Sci., 69: 1, 47-49.

GEORGE, C.F., BLACKWELL, E.W. and DAVIES, D.S. (1974).
J. Pharm. Pharmacol., 26, 265-267.

GIANELLY, R.E., VON DER GROEBEN, J.O., SPIVACK, A.P. and HARRISON, D.G. (1967).
N. Engl. J. Med., 277, 1215-1219.

GIBALDI, M., BOYES, R.N. and FELDMAN, S. (1974).
J. Pharm. Sci., 60, 1338-1340.

GIBLADI, M., NAGASHIMA, R. and LEVY, G. (1969).
J. Pharm. Sci., 58: 2, 193-197.

GIBALDI, M. and PERRIER, D. (1974).
Drug Metabolism Revs., 3: 2, 185-199.

GIBALDI, M. and PERRIER, D. (1975).
Pharmacokientics. Marcel Dekker Inc., N.Y.

GILLETTE, J.R. (1963).
Prog. Drug Res., 6, 11-73.

GOLDBERG, A.H. (1970).
Int. Anaesthesiol. Clin., 8, Summer, 1970, 315-327.

GRAFFNER, C., CONRADSON, T.B., HOFVENDAHL, S. and RYDEN, L. (1979).
Clin. Pharmacol. Ther., 27: 1, 64-71.

GREENBLATT, D.J., BOLOGNINI, V., KOCH-WESER, J. and HARMATZ, J.S. (1976).
JAMA, 236, 273-277.

GREENBLATT, D.J. and KOCH-WESER, J. (1975).
N. Engl. J. Med., 293: 14, 702-705.

GREENWAY, C.V. and STARK, R.D. (1971).
Physiol. Revs., 51, 23-65.

GROSSMAN, J.I., COOPER, J.A. and FRIEDEN, J. (1969).
Am. J. Cardiol., 24, 191-197.

GROSSMAN, J.L., LUBOW, L.A., FRIEDEN, J. and RUBIN, I.L. (1968).
Arch. Intern. Med., 121, 396-401.

HALKIN, H., MEFFIN, P., MEHNON, K.L. and ROWLAND, M. (1975).
Clin. Pharmacol. Ther., 17, 669-676.

HARRIS, A.S., AGUIRRE, Y., GUERRA, LIPTAK, R.A. and BRIGHAM, J.C. (1955/56).
J. Appl. Physiol., 8, 499-503.

HARRISON, D.C. and ALDERMAN, E.L. (1972).
Mod. Treat., 9, 139-175.

HARRISON, D.C., SPROUSE, J.H. and MORROW, A.G. (1963).
Circulation, 28, 486-491.

HEINONEN, J., TAKKI, S. and JARHO, L. (1970).
Acta anaesth. Scandinav., 14, 89-95.

HILEY, C.R. and YATES, M.S. (1978).
Clin. Sci. Molecular Med., 55, 001-004.

HOLLUNGER, G. (1960a).
Acta pharmacol. et toxicol., 17, 356-364.

HOLLUNGER, G. (1960b).
Acta pharmacol. et toxicol., 17, 365-373.

INVESTIGATOR'S BROCHURE TOCAINIDE HCl (1977).
Astra Pharmaceutical Ltd.

ISHIZAKI, T., TAWARA, K. and ORINCHI, K. (1977).
Adv. Clin. Pharmacol., 13, 265-282.

JEWIT, D.E., KISHON, Y. and THOMAS, M. (1968).
Lancet, 1, 266-270.

KAIHARA, S., VAN HEERDEN, P.D., MIGITA, T. and WAGNER, H.N. (1968)
J. Appl. Physiol., 25, 696-700.

KAO, F.F. and JALAR, U.H. (1959).
Brit. J. Pharmacol., 14, 522-526.

KATZ, J. (1968).
Anaesthesiology, 29: 2, 249-253.

KEENAGHAN, J.B. (1968).
Anaesthesiology, 29, 110-112.

KEENAGHAN, J.B. and BOYES, R.N. (1972).
J. Pharmacol. Exp. Ther., 180: 2, 454-463.

KETTERER, S.G., WIEGLAND, B.D. and RAPPAPORT, E. (1960).
Am. J. Physiol., 199, 481-484.

KORNAUSER, D.M., WOOD, A.J., VESTAL, R.E., WILKINSON, C.R. and
SHAND, D.G. (1978).
Clin. Pharmacol., Ther., 23: 2, 165-174.

LALKA, D. and FELDMAN, H. (1974).
J. Pharm. Sci., 63: 11, 1812.

LALKA, D., MANIOU, V., BERLIN, A., BAER, D.T., DODD, B. and
MAYER, B. (1976a).
Clin. Pharmacol. Ther., 19, 110.

LALKA, D., Meyer, M.B., DUCE, B.R. and ELVIN, A.T. (1976b).
Clin. Pharmacol. Ther., 19: 6, 757-766.

LAUTT, W.W. and SKELTON, F.S. (1976).
Can. J. Physiol. Pharmacol., 55: 1, 7-12.

LOWN, B., FAKHRO, A.M., HOOD, W.B. and THRON, G.W. (1967).
JAMA, 199: 3, 188-198.

LOWRY, O.H., ROSEBOROUGH, N.J., FARR, A.L. and RNADALL, R.J. (1951).
J. Biol. Chem., 193, 265-275.

LOO, J. and RIEGELMAN, S. (1968).
J. Pharm. Sci., 57: 6, 918-928.

MANDEL, W.J. and BIGGER, J.T. (1971).
J. Pharmacol. Exp. Ther., 178: 1, 81-93

MATHER, L.E. and THOMAS, J. (1972).
Life Sci., 11, 915-919.

MATHER, L.E. and TUCKER, G.T. (1974).
J. Pharm. Sci., 63: 2, 306-307.

MCDEVITT, D.G. and NIES, A.S. (1976).
Cardio. Res., 10, 494-498.

MCDEVITT, D.G., NIES, A.S., WILKINSON, G.R., SMITH, R.F.,
WOOSLEY, R.L. and OATES, J.A. (1976).
Clin. Pharmacol. Ther., 19: 4, 396-402.

MAYERSOHN, M. and GIBLADI, M. (1971).
Am. J. Pharm. Ed., 35, 19-28.

MENDELL, P.L. and HOLLENBERG, N.K. (1971).
Am. J. Physiol., 221: 6, 1617-1620.

MOLDEUS, P., GRUNDIN, R., VON BAHR, C. and ORRENIUS, S. (1973).
Biochem. Biophysic. Res. Comm., 55: 3, 937-944.

MÖLLER, E., MCINTOSH, J.F. and VAN SLYKE, D.D. (1929).
J. Clin. Invest., 6, 427-465.

MOORE, E.N. and SPEAR, J.F. (1973).
The electrophysiological effects of Astra W36095 on the
heart. Univ. of Pennsylvania, 6th July, 1973.

NAGASHIMA, R., LEVY, G. and O'REILLY (1968).
J. Pharm. Sci., 57: 1, 188-1895.

NATION, R.L. and TRIGGS, E.J. (1977).
Br. J. Clin. Pharmacol., 4, 439-448.

NATION, R.L., TRIGGS, E.J. and SELIG, M. (1977).
Br. J. Clin. Pharmacol., 4, 439-448.

NASH, T. (1953).
Biochem. J., 55, 416-421.

NELSON, E. (1961).
J. Pharm. Sci., 50, 181-192.

NEUTZE, J.M., WYLER, F. and RUDOLPH, A. (1968).
Am. J. Physiol., 215, 486-495.

NIES, A.S., EVANS, G.H. and SHAND, D.G. (1973).
Am. Heart, J., 85: 1, 97-102.

NIES, A.S., WILKINSON, G.R., RUSH, B.D., STROTHER, J.T. and MCDEVITT, D.G. (1976).
Biochem. Pharmacol., 25, 1991-1993.

NOTARI, R.E. (1975).
Biopharmaceutics and Pharmacokinetics. An introduction 2nd Ed. Marcel Dekker Inc., N.Y.

NYBERG, G., KARLEN, B., HEDLUND, I., GRUNDIN, R. and VON BAHR, C. (1977).
Acta pharmacol. et toxicol., 40, 337-346.

OHNHAUS, E.E. (1979).
Br. J. Clin. Pharmacol., 7, 223-229.

OHNHAUS, E.E., EMOUS, E. and BRECKENRIDGE, A. (1970).
Pflügers ARch., 323, 182-186.

OHNHAUS, E.E. and LOCHER, J.T. (1975).
Eur. J. Pharmacol., 31, 161-165.

OHNHAUS, E.E., THORGURSSON, S.S., DAVIES, D.S. and BRECKENRIDGE, A. (1971).
Biochem. Pharmacol., 20, 2561-2570.

OMURA, T. and SATO, R. (1964).
J. Biol. Chem., 239: 7, 2370-2378.

OMURA, T., SATO. R., COOPER, D.Y., ROSENTHAL, O. and ESTABROOK, R.W. (1965).
Fed. Proc., 24, 1181-1189.

ORRENIUS, S. and ERNSTER, L. (1964).
Biochem. Biophys. Res. Comm., 16: 1, 60-65.

PANG, K.S. and ROWLAND, M. (1977a).
J. Pharmacokinet. Biopharm., 5: 6, 625-653.

PANG, K.S. and ROWLAND, M. (1977b).
J. Pharmacokinet. Biopharm, 5: 6, 655-679.

PERRIER, D. and GIBALDI, M. (1972).
J. Clin. Pharmacol., 12, 449-451.

PERRIER, D. and GIBALDI, M. (1973).
J. Pharmacokinet. Biopharm., 1, 16-22.

PERRIER, D. AND GIBALDI, M. (1974).
J. Pharmacol. Exp. Ther., 191: 1, 17-24.

PERUCCA, E. and RICHENS, A. (1979).
Br. J. Clin. Pharmac., 8, 21-31.

PFEIFER, H.J., GREENBLATT, D.J. and KOCH-WESER, J. (1976).
Am. Heart J., 92, 168-173.

REMMER, H. (1972).
Europ. J. Clin. Pharmacol., 5, 116-136.

RIEGELMAN, S., LOO, J. and ROWLAND, M. (1968).
J. Pharm. Sci., 57: 1, 128-133.

ROWLAND, M. (1972a).
Eur. J. Pharmacol., 17, 352-356.

ROWLAND, M. (1972b).
J. Pharm. Sci., 61, 70-74.

ROWLAND, M., BENET, L.Z. and GRAHAM, G.G. (1973).
J. Pharmocokinet. Bipharm., 1: 2, 123-136.

ROWLAND, M., THOMSON, P.D., GUICHARD, A. and MELMON, K.L. (1971).
Ann. N.Y. Acad. Sci., 179, 383-397.

ROWELL, L.B., BLACKMON, J.R., MARTIN, R.H., MAZZARELLA, J.A. and BENCE, R.A. (1965).
J. Appl. Physiol., 20, 384-394.

RUDOLPH, A.M. and HEYMANN, M.A. (1967).
Circ. Res., 21, 163-184.

SAPIRSTEIN, L.A. (1956).
Circ. Res., 4, 689-692.

SASAKI, Y. and WAGNER, H.N. (1971).
J. Appl. Physiol., 30: 6, 879-884.

SCHARTZ, R.D., SIDELL, F.R. and CUCINELL, S.A. (1974).
J. Pharmacol. Exp. Ther., 188, 1-7.

SCHWARTZ, M., COVINO, B., DUCE, B., NARANG, R., FIORE, J., MARKELIS, M., RIZVI, S. and SMITH, E. (1979).
J. Clin. Pharmacol., 19, 100-107.

SCHUMACHER, R.R., LIEBERSON, A.D., CHILDRESS, R.H. and WILLIAMS, J.F. (1968).
Circulation, 37, 965-972.

SHAND, D.G., KORNHAUSER, D.M. and WILKINSON, G.R. (1975).
J. Pharmacol. Exp. Ther., 195: 3, 424-432.

SHAND, D.G., NUCKOLLS, E.M. and OATES, J.A. (1970).
Clin. Pharmacol. Ther., 11, 112-120.

SHAND, D.G. and RANGNO, R.E. (1972).
Pharmacology (Basel), 7, 159-163.

SHERIDAN, D.J., RAWLINS, M.D., CRAWFORD, L. and JULIAN, D.G. (1977).
Lancet, 16, 824-825.

SIMPSON, A.M., ERZOW, L. and SAPIRSTEIN, L.A. (1954).
Am. J. Physiol., 177, 319-324.

SINGH, J.B. and KOCOT, S.L. (1976).
Am. Heart J., 91, 430-436.

SINGH, B.N. and VAUGHAN WILLIAMS, E.M. (1971).
Cir. Res., 29, 286-295.

SMITH, E.R. (1972).
Astra Report No: 423 W53.

SMITH, E.R. and DUCE, B.R. (1971).
J. Pharmacol. Exp. Ther., 179: 3, 580-585.

SOUTHWORTH, J.L., MCKUSICK, V.A., PEIRCE, E.C. and RAWSON, F.L. (1950).
JAMA, 143, 717-720.

STANNARD, M., SLOMAN, G. and SANGSTER, L. (1968).
Brit. Med. J., 2, 468-469.

STENSON, R.E., CONSTANTINO, R.T. and HARRISON, D.C. (1971).
Circulation, 43, 205-211.

STRONG, J.M., MAYFIELD, D.E., ATKINSON, A.J., BURRIS, B.C., RAYMON, F. and WEBSTER, L.T. (1975).
Clin. Pharmacol. Ther., 17: 2, 184-194.

STROTHER, A., SOONG, S.L., DEV, V. and SADRI, M. (1977).
Live Sci., 21: 1, 71-82.

STRONG, J.M., PARKER, M. and ATKINSON, A.J. (1973).
Clin. Pharmacol. Ther., 14: 1, 67-72.

SUNG, C.Y. and TRUANT, A.P. (1954).
J. Pharmacol. Exp. Therap., 112, 432-443.

THOSMON, P.D., MELMON, K.L., RICHARDSON, J.A., COHN, K., STEINBRUNN, W., CUDIHEC, R. and ROWLAND, M. (1973).
Ann. Int. Med., 78: 4, 499-508.

TOBIAS, C.A., JONES, H.B., LAWRENCE, J.H. and HAMILTON, J.G. (1949).
J. Clin. Invest., 28, 1375-1385.

TUCKER, G.T. and BOAS, R.A. (1971).
Anaesthesiology, 34: 6, 538-549.

TUCKER, G.T., BOYES, R.N., BRIDENBAUGH, P.O. and MOORE, D.C. (1970).
Anaesthesiology, 33, 287-303.

WAGNER, J.G. (1974).
Clin. Pharmacol. Ther., 16: 4, 691-700.

WAGNER, J.G. (1975).
Fundamentals of Clinical Pharmacokinetics
Drug Intelligence Publications, Hamilton, Ill.

WAGNER, J.G., NORTHAM, J.I., ALWAY, C.D. and CARPENTER, O.S. (1965).
Nature, 207, 1301-1302.

WAGNER, H.N., RHODES, B.A., SASAKI, Y. and RYAN, J.P. (1969).
Invest. Radiol, 4, 374-386.

WATSON, (1949).
Modern theories of Organic Chemistry. 2nd Ed.
Oxford University Press, London.

Wilkinson, G.R. (1975).
Ann. Rev. Pharmacol., 15, 11-27.

WILKINSON, G.R. and SCHENKER, S. (1975).
Drug Metabolisms Revs., 4: 2, 139-175.

WILKINSON, G.R. and SCHENKER, S. (1976).
Biochem. Pharmacol., 25, 2675-2681.

WILKINSON, G.R. and SHAND, D.G. (1975).
Clin. Pharmacol. Ther., 18: 4, 377-390.

WILLIAMS, C.H. and KAMIN, H. (1962).
J. Biol. Chem., 237: 2, 587-595.

WINKLE, R.A., ANDERSON, J.L., PETERS, F., MEFFIN, P.J., FOWLES, R.E. and HARRISON, D.C. (1978).
Circulation, 57, 787-792.

WINKLER, K., BASS, L., KEIDING, S. and TYGSTRUP, N. (1974).
Alfred Benson Symposium VI: Regulation of Hepatic Metabolism, In F. Lundguist and N. Tygstrup (eds.)
Munksgaard, Copenhagen.

WINKLER, K., KEIDING, S. and TYGSTRUP, N. (1973).
The Liver: Quantitative Aspects of Structure and Functions
In P. Paumgartner and R. Presig (eds.)
Karger, Basel.

WINKLE, MEFFIN, P.J., FITZGERALD, J.W. and HARRISON, D.C. (1976).
Circulation, 54, 884-889.

YATES, M.S., HILEY, C.R. and BACK, D.J. (1979).
Life Sci., 24, 535-540.

ZITO, R.A., REID, P.R. and LONGSTRETH, J.A. (1977).
Am. Heart J., 94: 3, 292-296.